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STRUCTURAL STUDIES OF PLANT GUM FROM SAP OF THE LAC TREE, *Rhus vernicifera*

RYUICHI OSHIMA AND JU KUMANOTANI*
Institute of Industrial Science, University of Tokyo, 7-22-1, Roppongi, Minatoku, Tokyo 106 (Japan)
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ABSTRACT

The plant gum isolated from sap of the lac tree, *Rhus vernicifera* (China), was separated into two fractions having mol. wt. 84,000 and 27,700 by aqueous-phase gel-permeation chromatography. The fractions contain D-galactose (65 mol%), 4-*O*-methyl-D-glucuronic acid (24 mol%), D-glucuronic acid (3 mol%), L-arabinose (4 mol%), and L-rhamnose (3 mol%). Smith degradation of the carboxyl-reduced polysaccharides gives products of halved molecular weight, and these consist of a β-(1→3)-linked galactopyranan main chain and side chains made up of galactopyranose residues. Peripheral groups, such as α-D-Gal*p*-, α-D-Gal*p*-(1→6)-β-D-Gal*p*-, 4-*O*-methyl-β-D-Glc*p*A-, and 4-*O*-methyl-β-D-Glc*p*A-(1→6)-β-D-Gal*p*-, are attached to this interior core through β-(1→3)- or β-(1→6)- linkages.

INTRODUCTION

Japanese lacquer is made from sap of the lac tree, *Rhus vernicifera* (Japan, China, and Korea), and has been used in Asian countries for thousands of years as a superdurable coating material. The sap consists of urushiol (60–65%)[1–3], gummy substance (4%), nitrogenous material (1%), and water (30%)[4,5].

We have recently demonstrated that the gummy substance and the nitrogenous material play a significant role in making the characteristic architecture of Japanese lacquer coatings, and are concerned with the drying process[6].

Only limited knowledge is available concerning the plant gum that is the major constituent of the gummy substance. Oda *et al.* reported that D-galactose, D-xylose, L-arabinose, L-rhamnose, D-galacturonic acid, D-glucuronic acid, and 6-*O*-galactosyluronic-galactose were found in the acid-hydrolyzate of the gum fractionated from the gummy substance by precipitation with ethanol[7,8]. However, ambiguities remain in the experimental results, as isolation and identification of the gum and mono- and di-saccharides derived therefrom were unsatisfactory.

*Present address: Ehime University, 3 Bunkyocho, Matsuyama, Ehime 790, Japan.

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We have succeeded in isolating the nitrogen-free plant gum, which was resolved into two fractions by aqueous-phase gel-permeation chromatography. These were examined by sugar and deuteriomethylation analyses, Smith degradation, and partial acid hydrolysis. From these results, the structure of the gum is discussed.

RESULTS AND DISCUSSION

Isolation of the gum. — The water-soluble portion of the acetone powder separated as acetone-insoluble material from the sap of *Rhus vernicifera* contains mono- and oligo-saccharides (~20%), a trace of glycoproteins (laccase[9], stellacyanin[10] etc.), and the gum under consideration (80%), which is present as salts with calcium, magnesium, and sodium ions in the molar ratio of 8:5.5:3. Substances of lower molecular weight were removed by extensive dialysis. The remaining material was subjected to cation-exchange chromatography to remove the proteins; the nitrogen-free, acid-form gum was obtained in the void volume. This product is of bimodal molecular-weight distribution, and was resolved into two fractions (FR1 and FR2) by preparative aqueous-phase gel-permeation chromatography (g.p.c., Fig. 1) as the salt form. Characteristics of each fraction are listed in Table I. They showed an i.r. absorption band at 1615 cm^{-1} (carboxylate group), which shifted to 1740 cm^{-1} upon acidification. An *O*-methyl resonance (δ 3.38) and resonances arising from anomeric protons of β-linked D-glycopyranosyl residues (δ 4.4–4.6) were observed in the ^{1}H-n.m.r. spectra in D_2O. In the ^{13}C-n.m.r. spectrum in 0.1M sodium chloride in D_2O (Fig. 2), signals for carbonyl carbon (δ 175), C-1 of β-(1→3)- and β-(1→6)-linked D-glycopyranosyl residues (δ 104.1, 103.2), glycosyloxy-substituted C-3 (δ 82.3), unlinked C-6 (δ 60.9, 61.5, 62.0), and *O*-methyl carbon (δ 60.3) were recognized. Weak signals at δ 101.3 and 101.6 may be

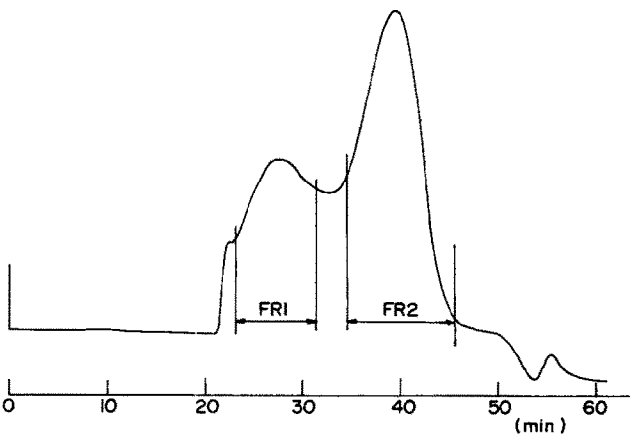


Fig. 1. Separation of plant gum into two fractions (FR1 and FR2) by high-performance gel chromatography. Conditions: column, TSK-gel G3000SW (60 × 2.2 cm); eluent, 67mM phosphate buffer (pH 6.98); flow rate, 4 mL/min; detection, r.i. A solution of plant gum (120 mg) in the eluent (3 mL) was injected onto the column. The regions marked by arrows were collected

TABLE I

CHARACTERISTICS OF PLANT GUM FROM SAP OF THE LAC TREE *(Rhus Vernicifera)*

Fraction	*Molecular weight* $\times 10^{-4}$			$[\alpha]_{589}^{26}$ (c, H_2O)	pKa_{app}	$[CO_2H]$ equiv. $\times 10^4$/g
	M_w	M_n	M_w/M_n			
FR1	8.40			−0.8° (0.6)	3.07	12.5
FR2	2.77	2.22	1.22	+8.7° (0.6)	3.07	11.9

TABLE II

CONSTITUENTS OF PLANT GUM FROM SAP OF THE LAC TREE *(Rhus vernicifera)*

Sugar	*Content (mol%)*		$M^+ + 1 - 60^a$
	FR1	*FR2*	
D-Galactose	65.1	67.0	375
4-*O*-Methyl-D-glucuronic acid	24.5	23.6	349
D-Glucuronic acid	3.3	2.6	377
L-Arabinose	4.8	5.2	289
L-Rhamnose	2.0	4.1	317

[a]Base peaks in c.i. mass spectra of alditol acetates of the hydrolyzate of the carboxyl-reduced polysaccharides made with $NaBD_4$.

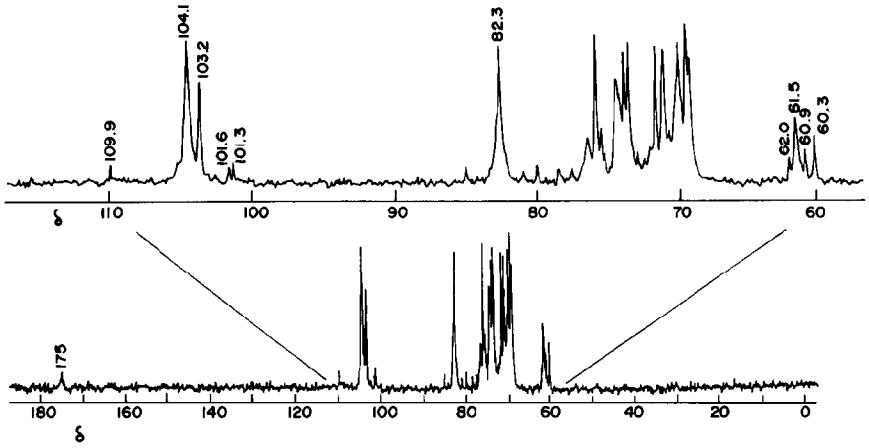


Fig. 2. 75-MHz ^{13}C-n.m.r. spectrum of native FR2 in 0.1M sodium chloride in D_2O.

assigned to C-1 of α-linked D-glycopyranosyl residues[11]. A weak signal was observed at δ 109.9.

Constituent monosaccharides. — In the reduced and acetylated hydrolyzate of the carboxyl-reduced polysaccharide prepared[12] with $NaBD_4$, acetates of rhamnitol, arabinitol, 4-*O*-methylglucitol, glucitol, and galactitol were identified. The

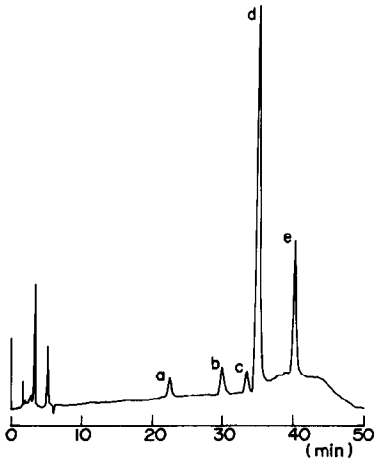


Fig. 3. L.c. diagram of diastereoisomeric 1-(*N*-acetyl-L-α-methylbenzylamino)-1-deoxyalditol acetates derived from the acid-hydrolyzate of the carboxyl-reduced plant gum (FR1). Conditions: column, silica gel (Develosil 60-3, 3 μm, 150 × 4.6 mm); eluent, linear gradient from 97:3 hexane–ethanol to 95:5 in 40 min, maintained at the final concentration for 5 min, and reset to the initial concentration after 5 min; detection, u.v. 210 nm. Peaks: diastereoisomers of (*a*) L-rhamnose, (*b*) L-arabinose, (*c*) D-glucose (*d*) D-galactose, and (*e*) 4-*O*-methyl-D-glucose.

TABLE III

METHYLATION ANALYSIS OF CARBOXYL-REDUCED AND SMITH-DEGRADED PLANT GUM FROM SAP OF THE LAC TREE (*Rhus vernicifera*)

	Methylated sugars[a]	*Mol%*		
		C.r.[b]	*SI*	*SII*
FR1	2,3,4-Rha	1.6 (0.4)	0.0	0.0
	2,3,5-Ara	2.2 (0.6)	0.0	0.0
	2,3,4,6-Glc[c]	18.9 (5)	0.0	0.0
	2,3,4,6-Gal	9.3 (2)	23.8 (4)	14.4 (1.8)
	2,4,6-Gal	20.5 (5)	55.2 (9)	72.6 (9)
	2,3,4-Gal	17.5 (5)	5.4 (1)	5.8 (0.7)
	2,4-Gal	26.3 (7)	14.2 (2.5)	7.2 (1)
FR2	2,3,4-Rha	1.3 (0.3)	0.0	0.0
	2,3,5-Ara	2.4 (0.6)	0.0	0.0
	2,3,46-Glc[c]	18.9 (5)	0.0	0.0
	2,3,4,6-Gal	9.3 (2)	16.1 (2)	11.7 (1)
	2,4,6-Gal	15.1 (4)	64.2 (8)	81.8 (8)
	2,3,4-Gal	23.8 (6)	5.0 (0.6)	0.0
	2,4-Gal	26.9 (7)	15.0 (2)	5.9 (0.6)

[a]2,3,4,6-Gal = 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-galactitol, etc. [b]Carboxyl-reduced polysaccharide. [c]This is derived from terminal 4-*O*-methyl-D-glucuronic acid (90%) and terminal D-glucuronic acid (10%) groups.

values for the $M^+ + 1 - 60$ ions in the c.i. mass spectra, and e.i.–m.s. analysis, showed the acetates of 4-*O*-methylglucitol and glucitol to be substituted by two deuterium atoms at C-6, indicating that these were derived from corresponding uronic acid residues. Galacturonic acid residues were absent; this result contradicts the analysis by Oda *et al.*[7].

In the liquid chromatogram of the acetylated products of reductive amination of the hydrolyzate with L-(-)-α-methylbenzylamine in the presence of $NaBH_3CN$ (Fig. 3)[16,17] diastereoisomers of L-arabinose, L-rhamnose, D-glucose, D-galactose, and 4-*O*-methyl-D-glucose were identified. In Table II are listed the monosaccharide compositions of FR1 and FR2.

Deuteriomethylation analysis. — In the left column of Table III are summarized the results of linkage analysis of fully deuteriomethylated[18], carboxyl-reduced gum. D-Galactose residues are in the pyranose form and are located at non-reducing ends or are (1→3)-, (1→6)-, and 1,3,6-linked. L-Arabinosyl groups are in the furanose form, L-rhamnosyl and 4-*O*-methyl-D-glucosyluronic acid groups are in the pyranose form, and all of them are located at terminal, non-reducing ends[19].

In the mass spectrum of the alditol acetate from the terminal 4-*O*-methyl-D-glucuronic acid group (Fig. 4), peaks originating in terminal D-glucuronic acid are detected as primary (*m/z*, 214) and secondary fragments (*m/z*, 132 and 154), which may arise according to the fragmentation reactions[20] shown in Scheme 1. The intensities of these fragments relative to corresponding peaks from 4-*O*-methyl-D-glucuronic acid were 8.0, 17.8, and 6.1% for the ions of *m/z* 214, 132 and 154, respectively. As the content of D-glucuronic acid is ~10% of that of the 4-*O*-methylated analogue, it is also in the pyranose form and occupies non-reducing ends.

Anomeric nature of sugar residues. — The previously mentioned ^{13}C- and ^1H-n.m.r. data and the low specific rotation (Table I) of the native gum indicate that

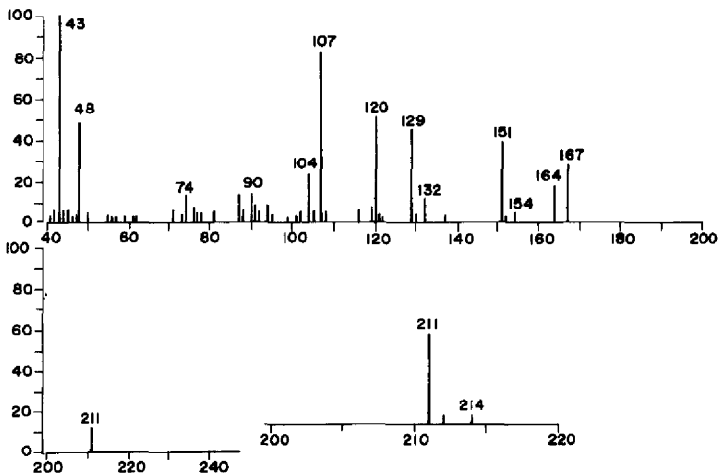
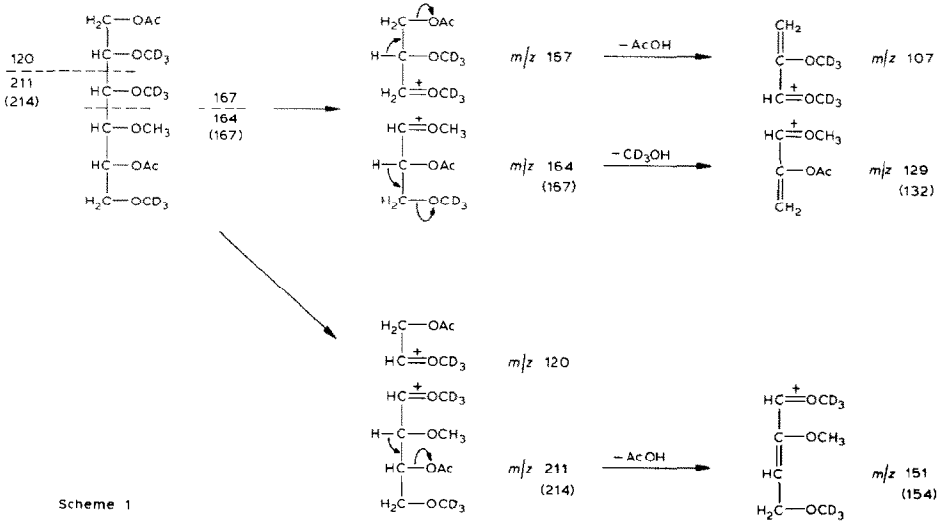


Fig. 4. Mass spectrum of partially deuteriomethylated alditol acetates from the terminal, nonreducing 4-*O*-methyl-D-glucuronic acid and D-glucuronic acid groups.



Scheme 1

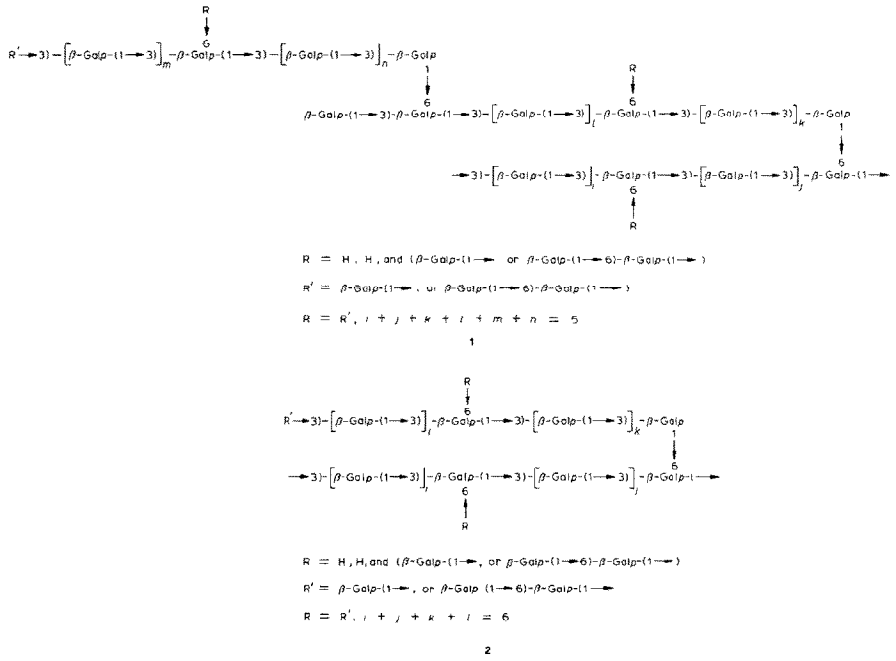
TABLE IV

WEIGHT-AVERAGE AND NUMBER-AVERAGE MOLECULAR WEIGHTS OF PLANT GUM FROM *Rhus vernicifera* AND ITS DERIVATIVES

	FR1	*FR2*		
	M_w	M_w	M_n	M_w/M_n
Native	84,000	27,700	22,600	1.22
Carboxyl-reduced	73,000	20,800	16,600	1.25
SI	45,000	12,000	9,500	1.25
SII	36,000	8,700	7,200	1.22

most sugar residues are β-linked. Only 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol was detected upon methylation analysis of the chromium trioxide-oxidized[21] carboxyl-reduced polysaccharides, indicating that some of the D-galactopyranose residues are α-linked, whereas other galactopyranose residues, terminal 4-*O*-methyl-D-glucopyranuronic acid, terminal D-glucopyranuronic acid, and terminal L-rhamnopyranose units are β-linked. A low-field signal (δ 109.9) observed in Fig. 2 may be due to C-1 of α-L-arabinofuranosyl residues[11].

Smith degradation of carboxyl-reduced polysaccharide. — Smith degradation[22] of the carboxyl-reduced polysaccharides from FR1 and FR2 gave degraded polysaccharides (SI) in respective yields of 63% (FR1) and 46% (FR2); these were submitted further to a second degradation giving polysaccharides designated as SII. Their molecular weights are compared in Table IV; the slightly larger, apparent values for the native gums than those for carboxyl-reduced samples may arise from the polyelectrolyte nature of the former. It should be noted that molecular-weight distribution indices for SI and SII remained intact.



From the results of methylation analysis of SI and SII (Table III, columns 2 and 3), structure **1** may be taken as a mean repeating-unit of SI from FR1. On the second degradation, **1** would give a polysaccharide composed of (1→3)-, (1→6)-, 1,3,6-linked, and nonreducing galactopyranose residues in the ratio of 9:1:1:1. These values are close to those found for SII.

Similarly, **2** was assigned a mean repeating-unit of SI from FR2. Terminal and (1→6)-linked residues in **1** and **2** are substituted by periodate-labile groups.

Partial hydrolysis of FR2: separation of the hydrolyzate by reversed-phase l.c. — Native FR2 (67 mg) was partially hydrolyzed and then subjected to preparative gel-chromatography[23] to separate the monosaccharide (10 mg) and oligosaccharide portions (43 mg).

The former was composed of rhamnose (16%), arabinose (22%), and galactose (62%); the contents of rhamnose and arabinose in the whole hydrolyzate were estimated to be 3.0 and 4.2%, respectively. As those of L-rhamnose and L-arabinose in native FR2 are 4.1 and 5.2%, almost all residues of these sugars were liberated during the mild hydrolysis.

Portions of the oligosaccharides were reduced by the Taylor–Conrad procedure[12] and the products resolved by gel chromatography[23] followed by reversed-phase (r.p.) l.c. on ODS-silica gel columns with water as eluent[24] (Fig. 5).

Methylation analysis showed oligomer A in Fig. 5b to be D-Gal*p*-(1→3)-D-Gal-ol. Oligomer B in Fig. 5a and b showed an *O*-methyl resonance (δ 3.50) and a doublet for a β-linked anomeric proton (δ 4.43, J 7.5 Hz) in the ^{1}H-n.m.r. spec-

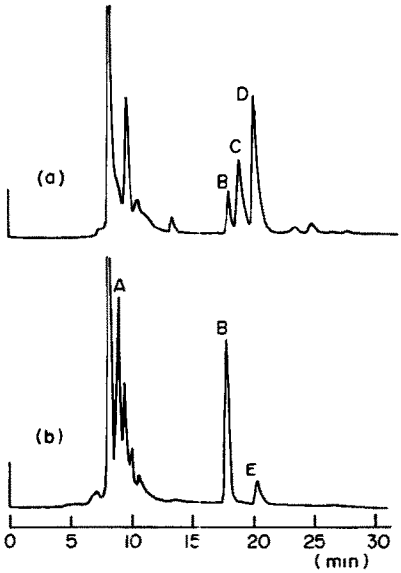


Fig. 5. Reversed-phase l.c. diagram of (*a*) second and (*b*) third fractions in the gel chromatogram (see experimental section) of the reduced acid-hydrolyzate of native gum (FR2). Conditions: column, ODS-silica gel (Develosil ODS-5, 5 μm, 250 × 8 mm × 2); eluent, water; flow rate, 2.5 mL/min; detection, r.i. Peaks were identified by methylation analysis as described in the text.

trum; it was assigned as 4-*O*-methyl-β-D-Glc*p*-(1→6)-D-Gal-ol after methylation analysis. It is clearly shown that the presence of *O*-methyl groups considerably increases capacity factors of solutes in r.p. l.c.

Methylation analysis showed oligomers C and E to be 4-*O*-methyl-D-Glc*p*-(1→6)-D-Gal*p*-(1→3)-D-Gal-ol and 4-*O*-methyl-D-Glc*p*-(1→6)-D-Gal*p*-(1→6)-D-Gal-ol, respectively. Peak D gave a complicated g.l.c. diagram on methylation analysis, which was interpreted as being given by a mixture of two oligosaccharides D and E. Oligomer D may be assigned the structure 4-*O*-methyl-D-Glc*p*-(1→6)-[D-Gal*p*-(1→3)-]-D-Gal-ol or 4-*O*-methyl-D-Glc*p*-(1→3)-[D-Gal*p*-(1→6)-]-D-Gal-ol.

Partial hydrolysis of FR2: separation of hydrolyzate as benzoates by l.c. — In the previous section, oligosaccharide-alditols containing *O*-methyl groups were well resolved by r.p. l.c., but those composed of galactose residues exhibited small capacity-factors and were incompletely separated; they were resolved as benzoates by liquid–solid chromatography (l.s.c.) and r.p.–l.c.[25].

The hydrolyzate obtained under the same conditions as in the previous section was separated into neutral and acidic fractions by anion-exchange chromatography. Each fraction was successively reduced, benzoylated, and applied onto a column of silica gel to give the chromatograms shown in Fig. 6.

Each peak was analyzed on an ODS-silica column (see Fig. 7). A fraction that contained several components was further resolved on a semi-preparative column. Purified products obtained from the acidic part of the hydrolyzate were examined by ^{1}H-n.m.r. to detect the *O*-methyl group.

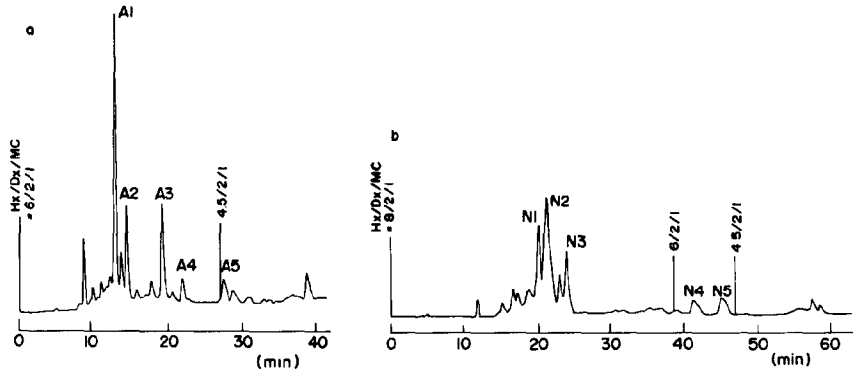


Fig. 6. Preparative separation of benzoates of oligosaccharide-alditols from the acidic (*a*) and the neutral (*b*) parts of the acid-hydrolyzate by liquid–solid chromatography. Conditions: column, Develosil 60-3, 3 μm, 250 × 8 mm; flow-rate, 2.5 mL/min; detection, 275 nm; eluent, hexane–1,4-dioxane–dichromethane mixtures, the composition was changed stepwise at marked positions as noted.

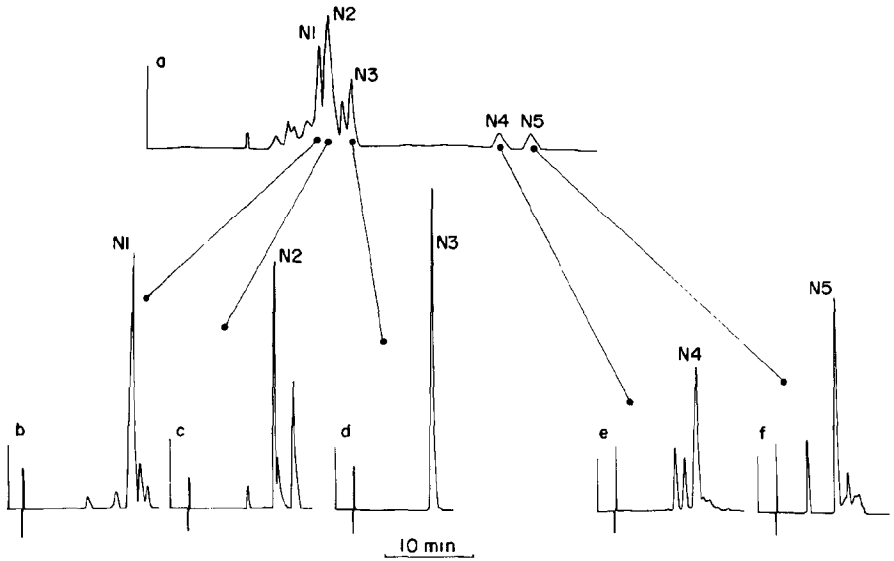


Fig. 7. Analysis of benzoates of oligosaccharide-alditols from the neutral part of the hydrolyzate of FR2 by liquid–solid chromatography and reversed-phase l.c. Chromatogram *a* is the same as Fig. 6b. Chromatograms *b–f* are reversed-phase l.c. curves of fractions N1–N5 of chromatogram *a*. Conditions for *b–f*: column, Develosil ODS-3, 150 × 4.5 mm; eluent, acetonitrile = 9:1 for *b–d*, 19:1 for *e* and *f*; flow rate, 1.25 mL/min; detection, 275 nm, 0.16 aufs.

The benzoates of oligosaccharide-alditols were directly converted into fully methylated products. Their anomeric configurations were determined by ^{1}H-n.m.r. spectroscopy. Retention times in g.p.c. clearly indicated the number of sugar residues in each oligomer. Glycosyl-linkage positions were determined by methylation analysis.

Consequently, we characterized several oligosaccharide-alditols (see Table V), whose structures are as follows:

4-*O*-Me-β-D-Glc*p*A-(1→6)-D-Gal-ol

3

β-D-Glc*p*A-(1→6)-D-Gal-ol

4

4-*O*-Me-β-D-Glc*p*A-(1→6)-β-D-Gal*p*-(1→3)-D-Gal-ol

5

4-*O*-Me-β-D-Glc*p*A-(1→6)-β-D-Gal*p*-(1→6)-D-Gal-ol

6

4-*O*-Me-β-D-Glc*p*A-(1→6)-β-D-Gal*p*-(1→6)-β-D-Gal*p*-(1→3)-D-Gal-ol

7

α-D-Gal*p*-(1→6)-D-Gal-ol

8

β-D-Gal*p*-(1→3)-D-Gal-ol

9

β-D-Gal*p*-(1→6)-D-Gal-ol

10

β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→3)-D-Gal-ol

11

β-D-Gal*p*-(1→6)-β-D-Gal*p*-(1→3)-D-Gal-ol

12

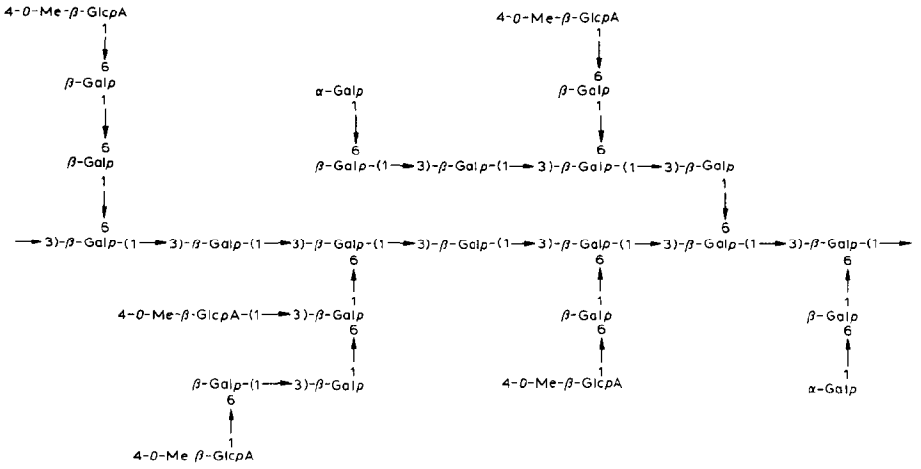


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TABLE V

CHARACTERISTICS OF OLIGOSACCHARIDE-ALDITOLS DERIVED BY PARTIAL HYDROLYSIS OF NATIVE GUM (FR2)

Peak in Fig. 6	*Structure*[a]	*Benzoates*					*Methylated derivatives*		
		Yield[b] *(mg)*	T_R *(min), eluant*[c] *L.s.c.*[d]	*R.p.l.c.*[e]	$[\alpha]_{589}^{20}$ *(c, $CHCl_3$)*	*M.p. (°)*	δ_{OCH_3}[f]	T_R *(min) g.p.c.*[g]	$\delta_{H\text{-}1}$[f] *($J_{1,2}$, Hz)*
A1	**3**	28	9.73[a]	9.30[c]	+31.7°		3.40	20.4	4.33 (7.5)
A2	**4**	11	11.60[a]	11.20[c]	+14 (0.32)	74–77			
A3	**5**	13	4.80[b]	8.76[d]	+45 (0.21)	92–94	3.46	19.4	4.30 (7.5), 4.28 (7.5)
A4	**6**	9	4.88[b]	9.07[d]	+37 (0.23)	73–75	3.45	19.2	4.30 (7.5), 4.27 (7.5)
A5	**7**	13	6.13[b]	*8.00*[e]	+41 (0.27)	111–114	3.60	18.5	4.36 (7.5), 4.27 (7.5)
N1	**8**	12	8.76[a]	15.00[c]	+68 (0.25)			20.1	5.20 (~3)
N2	**9**	17	9.23[a]	12.15[c]	+49 (0.85)	65–67		20.5	4.38 (7.5)
N3	**10**	8	10.61[a]	11.40[c]	+44 (0.56)	63–65		20.6	4.32 (7.5)
N4	**11**	6	4.78[b]	9.20[d]	+55 (0.20)	73–76		19.5	4.36 (7.5), 4.23 (7.5)
N5	**12**	5	4.86[b]	11.53[d]	+43 (0.14)	64–66		19.4	4.57 (7.5), 4.32 (7.5)

[a]See text. [b]From 424 mg of benzoates of the hydrolyzate for the acidic portion and from 200 mg for the neutral portion. [c]a, 8:2:1 (v/v) hexane–1,4-dioxane–dichloromethane; b, 9:4:2 (v/v) hexane–1,4-dioxane–dichloromethane; c, 9:1 acetonitrile–water; d, 19:1 acetonitrile–water; and e, 39:1 acetonitrile–water. [d]Conditions: column, Develosil 60-3, 150 × 4.5 mm; flow rate 1.25 mL/min; detection, 275 nm, 0.16 aufs. [e]Conditions: column, Develosil ODS-3, 150 × 4.5 mm; flow rate, 1.25 mL/min, detection 275 nm, 0.16 aufs. [f]Spectra of A1, A2, and N1–N3 were obtained with a JEOL MH-100 (100 MHz) spectrometer and those of A3-5, N4, and N5 with a JEOL GX-400 (400 MHz) spectrometer in $CDCl_3$. Chemical shifts are relative to the signal from internal $CHCl_3$ (δ 7.26). [g]Conditions: column, GELKO A-120 and A-110, 500 × 8 mm each; eluent, $CHCl_3$; flow rate, 1.0 mL/min; detection, r.i.

Structure of native plant gum. — Native FR2 is composed of the interior core **2** and peripheral groups, most of which contain terminal uronic acid groups.

Comparing the results of methylation analyses of the carboxyl-reduced polysaccharide and SI for FR2, it appears that all of the (1→3)-linked galactopyranose residues are situated in the interior core and (1→6)-linked residues in the peripheral portion. More than half of the β-(1→3)-linked galactopyranose residues and all of the terminal groups in the interior core are attached by periodate-labile groups through β-(1→6) and β-(1→3) linkages, respectively. Inspection of the structural fragments (**3**–**12**) revealed by partial hydrolysis indicates that 4-*O*-methyl-β-D-Glc*p*A-, 4-*O*-methyl-β-D-Glc*p*A-(1→6)-β-D-Gal*p*-, and α-D-Gal*p*-(1→6)-β-D-Gal*p*- constitute most of the peripheral groups.

A generalized structure **13** was constructed for the native FR2, assuming *i* =

3, $j = 2$, $k = 0$, and $l = 1$ in **2**, where terminal groups may occasionally be replaced by β-L-rhamnopyranose, β-D-glucopyranuronic acid, and α-L-arabinofuranose.

The contents of constituent monosaccharides and their linkages in FR1 are almost same as those of FR2. Both fractions (FR1 and FR2) may have similar overall structures, and differ only slightly in the side chain of the interior core.

EXPERIMENTAL

General methods. — Gas–liquid chromatography was performed with a Hitachi 063 gas chromatograph equipped with a flame-ionization detector and fused-silica, capillary columns (FFAP, 0.25 mm × 25 m, Gasukuro Kogyo, Tokyo). Peak areas were determined by a Chromatopac-ElA integrator (Shimazu, Kyoto) and converted into molar ratios on an effective carbon-response basis[26]. G.l.c.–m.s. was conducted with a Hitachi M-80 mass spectrometer equipped with a M-003 data processor. The ionization voltage was 70 and 100 eV for electron-impact and chemical ionization (ionizing gas, isobutane), respectively. The spectrum of Fig. 4 was obtained with a Hewlett–Packard 5985B g.c.–m.s. system.

Liquid chromatography was performed on hand-made instruments. For chromatography with gradient elution, a Hewlett–Packard model 1084B instrument was employed. Molecular-weights of polysaccharides were estimated by acqueous-phase g.p.c. [TSK-gel G2000SW-G3000SW-G4000SW, 600 × 7.6 cm each; eluent, 67mM phosphate buffer (pH 6.98) standardized with dextrans of mol. wt. ($\times 10^{-3}$) = 150, 70, 40, 20. and 3 (Seikagaku Kogyo, Tokyo)]. Organic-phase g.p.c. was carried out on TSK-gel G2000H_8 columns (600 × 7.5 mm × 2) or GELKO A-120 and A-110 columns (Hitachi Chemicals, 500 × 8 mm) with chloroform as eluent. L.c. columns were made by Yamauchi's method[27].

Specific rotations were determined with a PM-101 automatic, digital polarimeter (Union Giken, Osaka). ^{1}H-N.m.r. spectra were recorded at 100 and 400 MHz with JEOL MH-100 and GX-400 spectrometers. ^{13}C-N.m.r. spectra were obtained with a CXP-300 spectrometer (resonance frequency, 75 MHz, Bruker) by courtesy of Dr. H. Saito (National Cancer Center, Research Institute, Tokyo).

Isolation of plant gum and its fractionation. — The sap of *Rhus vernicifera* D.C. China (Saito Co., Osaka) (1 part) was mixed with acetone (3 parts). The precipitate was collected on a glass filter (G3), washed with acetone, and dried *in vacuo*. A mixture of the precipitate (1 part) and water (10 parts) was stirred for 2 h at room temperature and filtered through a glass filter (G4). The filtrate was dialyzed against water in a Visking cellulose bag (20/32) until oligosaccharides could not be detected in the dialyzate by aqueous-phase g.p.c. The solution in the bag was evaporated to dryness <40° to give a greenish powder. A solution of the powder (1 g) in 20 mL of water was injected into a stainless-steel column (30 × 2 cm) packed with cation-exchange resin (Hitachi gel no. 3019s, H^+), and the column was eluted with water at a flow rate of 3.5 mL/min. A peak at the void volume was collected and evaporated to give 650–700 mg of the nitrogen-free plant gum as

a colorless powder, $[\alpha]_{589}^{26}$ +3.0° (*c* 1, water). A solution of the plant gum (120 mg) in 3 mL of 67mM phosphate buffer (pH 6.98) was injected into a preparative, aqueous-phase g.p.c. column (TSK-gel G3000SW, 60 × 2.2 cm) and elution was performed with the same buffer.

Preparation of carboxyl-reduced polysaccharide. — Native FR1 and FR2 were reduced by the Taylor–Conrad procedure[12]. If necessary, $NaBD_4$ was employed to label the uronic acid residues.

Sugar analysis. — Carboxyl-reduced polysaccharide (2 mg) made with $NaBD_4$ was successively hydrolyzed with M trifluoroacetic acid (2 mL) for 5 h at 100°, reduced with sodium borohydride, and acetylated to give the alditol acetates, which were analyzed by g.l.c. and g.l.c.–m.s.

To determine the configuration of the monosaccharides, 1-(*N*-acetyl-L-(−)-α-methylbenzylamino)-1-deoxyalditol acetates derived from 2 mg of the carboxyl-reduced polysaccharides were analyzed on a 3-μm silica gel column (Develosil 60-3, Nomura Kagaku, Seto, Japan, 150 × 4.6 mm)[17]. Detailed chromatographic conditions are described in the legend to Fig. 3.

Deuteriomethylation analysis. — Deuteriomethylation of the carboxyl-reduced polysaccharide (2.5 mg) was completed by two iterations of the Hakomori procedure[18]. The perdeuteriomethylated product was hydrolyzed with 90% formic acid for 28 h at 100°, reduced with sodium borohydride in M ammonium hydroxide in ethanol[28], and acetylated. The mixture of partially deuteriomethylated, partially methylated alditol acetates obtained was analyzed by g.l.c. and g.l.c.–m.s.

Oxidation of acetylated, carboxyl-reduced polysaccharide with chromium trioxide. — The acetylated, carboxyl-reduced polysaccharide (50 mg) was prepared by the literature method[20], followed by purification on a preparative g.p.c. column (TSK-gel G2000HG, 60 × 2 cm; eluent, chloroform). The derivative (10 mg) was oxidized with 30 mg of chromium trioxide and 2 mg of the product was hydrolyzed with M trifluoroacetic acid (0.5 mL) and analyzed as alditol acetates by g.l.c.[20]. The remaining portion, methylated by the Hakomori procedure, was hydrolyzed with 90% formic acid (1 mL) at 100° for 20 h and analyzed by g.l.c. as the partially methylated alditol acetates.

Smith degradation. — The carboxyl-reduced polysaccharide was subjected to the Smith degradation[22] to yield polysaccharide (SI); FR1 gave 8.4 mg and FR2 6.9 mg. A second Smith degradation was performed on SI, giving the polysaccharide SII. Both SI and SII were subjected to methylation analysis.

Partial hydrolysis: separation of hydrolyzate by reversed-phase l.c. — The acid-form gum (67 mg) was hydrolyzed with 0.5M trifluoroacetic acid (7 mL) for 35 min at 100° in a sealed tube. The monosaccharide portion (10 mg) separated on a gel-chromatography column (Hitachi gel no. 3019s, H, 2 × 60 cm)[23] with 0.5% formic acid as eluant was analyzed by g.l.c. of the derived alditol acetates. The oligosaccharide portion (42.6 mg) was successively reduced by the Taylor–Conrad procedure[12], deionized with columns of Dowex 50W × 8 (H^+) and Dowex 1 × 8 (OH^-) resins, and separated into three fractions [fr.1, 10.3 mg; fr.2 (trimer), 4.8

mg; and fr.3 (dimer), 3.5 mg] by the foregoing gel chromatography. Each fraction was chromatographed on reversed-phase columns (Develosil ODS-5, Nomura Chemicals, 250 × 8 mm × 2) with water as eluent. Separated peaks were subjected to methylation analysis.

Partial hydrolysis: l.c. separation of the hydrolyzate as benzoates by l.c. — Native FR2 (700 mg) was hydrolyzed with 0.5M trifluoroacetic acid (70 mL) for 35 min at 100° in a sealed tube. The hydrolyzate was separated into neutral (251 mg) and acidic (450 mg) fractions on a column (21 × 1.7 cm) of Dowex 1 × 2 ($CH_3CO_2^-$, 200–400 mesh) resin. The neutral fraction (251 mg) was reduced with sodium borohydride, and the acidic portion (350 mg) by the Taylor–Conrad procedure, where the excess of borohydride was decomposed with M hydrochloric acid. The salts formed were removed by ion-exchange resins [Dowex 1 × 8 (OH^-) and Dowex 50W × 8 (H^+)], and the reduced materials were benzoylated with 10% benzoyl chloride in dry pyridine (100 mL and 150 mL for the neutral and acidic portions, respectively) for 2 days at 60°. Methanol (10 mL) was added and the mixture was stirred for 30 min, evaporated, and vacuum-distilled at 65°. The residue was mixed with chloroform (50 mL) and washed with water (20 mL × 5). The organic layer was evaporated and applied to a preparative g.p.c. column (TSK-gel G2000HG, 60 × 2.2 cm × 2), which was eluted with chloroform to give benzoates of the reduced hydrolyzate.

From the neutral part, benzoates of monosaccharide- and oligosaccharide-alditols were obtained in respective yields of 660 (60%) and 200 mg (18%), and from the acidic portion those of oligosaccharide-alditols (42 mg, 27%) were recovered. The mixtures of benzoates of oligosaccharide-alditols thus obtained were resolved on a column of silica gel (Develosil 60-3, 3 μm, 250 × 8 mm) and on an ODS-silica column (Develosil ODS-3, 3 μm, 150 × 8 mm); chromatographic conditions are described in the legends to Figs. 6 and 7.

The separated benzoates were methylated by the Hakomori method with 2M sodium methylsulfinylmethanide (0.1 mL per mg of benzoate) and methyl iodide (0.2 mL per mg of benzoate). The Me_2SO and methyl benzoate formed were removed under diminished pressure, the product was partitioned in chloroform–water, the chloroform layer was evaporated, and the residue purified by g.p.c. (column, GELKO A-110 and A-120, 500 × 8 mm; eluent, chloroform). Methylated products were analyzed by ^{1}H-n.m.r. and methylation analysis.

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REFERENCES

1 R. MAJIMA, *Ber.*, 55 (1922) 172–191.
2 S. V. SUNTHANKER AND C. R. DAWSON, *J. Am. Chem. Soc.*, 76 (1954) 5070–5074.
3 Y. YAMAUCHI, R. OSHIMA, AND J. KUMANOTANI, *J. Chromatogr.*, 243 (1982) 71–84.
4 S. ISHIMATSU, *Mem. Manchester Library and Philosophical Society (London and Paris)*, (1879) 249.
5 Japanese Industrial Standard K5950.
6 J. KUMANOTANI, R. OSHIMA, M. ACHIWA, AND K. ADACHI, *Intern. Conservation Restoration Cultural Properties (Tokyo), 2nd*, (1978) Abstr. p. 60.
7 Y. ODA, T. KANBE, AND K. HONDA, *Nippon Nogeikagaku Kaishi*, 37 (1963) 132–136.
8 Y. ODA, T. KANBE, AND K. HONDA, *Nippon Nogeikagaku Kaishi*, 38 (1964) 64–70.
9 T. NAKAMURA, *Biochim. Biophys. Acta*, 30 (1958) 44–52.
10 J. PEISACH, W. G. LEVINE, AND W. E. BLUMBERG, *J. Biol. Chem.*, 242 (1967) 2847–2858.
11 P. A. J. GORIN, *Adv. Carbohydr. Chem. Biochem.*, 38 (1981) 13–104; P. A. J. GORIN, *Carbohydr. Res.*, 101 (1982) 13–20.
12 R. L. TAYLOR AND H. E. CONRAD, *Biochemistry*, 11 (1972) 1383–1388.
13 R. OSHIMA, Y. KUROSU, AND J. KUMANOTANI, *J. Chromatogr.*, 179 (1979) 376–389.
14 R. OSHIMA, A. YOSHIKAWA, AND J. KUMANOTANI, *J. Chromatogr.*, 213 (1981) 142–145.
15 M. MCNEIL AND P. ALBERSHEIM, *Carbohydr. Res.*, 56 (1967) 239–248.
16 R. OSHIMA AND J. KUMANOTANI, *Chem. Lett.*, (1981) 943–946.
17 R. OSHIMA, Y. YAMAUCHI, AND J. KUMANOTANI, *Carbohydr. Res.*, 107 (1982) 169–176.
18 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
19 H. BJORNDAL, C. G. HELLERQVIST, B. LINDBERG, AND S. SVENSSON, *Angew. Chem., Int. Ed. Engl.*, 9 (1970) 610–619.
20 H. BJORNDAL, B. LINDBERG, Å. PILOTTI, AND S. SVENSSON, *Carbohydr. Res.*, 15 (1970) 339–349.
21 J. HOFFMAN AND B. LINDBERG, *Methods Carbohydr. Chem.*, 8 (1980) 117–122.
22 J. K. HAMILTON, G. W. HUFFMAN, AND F. SMITH, *J. Am. Chem. Soc.*, 81 (1959) 2176–2178.
23 J. KUMANOTANI, R. OSHIMA, Y. YAMAUCHI, N. TAKAI, AND Y. KUROSU, *J. Chromatogr.*, 176 (1979) 462–464.
24 G. PALLA, *Anal. Chem.*, 53 (1981) 1966–1967.
25 P. E. DANIEL, D. F. DE FEIDES, I. T. LOTT, AND R. H. MCCLUER, *Carbohydr. Res.*, 97 (1982) 161–180.
26 D. P. SWEET, R. H. SHAPIRO, AND P. ALBERSHEIM, *Carbohydr. Res.*, 40 (1975) 217–225.
27 Y. YAMAUCHI AND J. KUMANOTANI, *J. Chromatogr.*, 210 (1981) 512–515.
28 B. S. VALENT, A. G. DARVILL, M. MCNEIL, B. K. ROBERTSON, AND P. ALBERSHEIM, *Carbohydr. Res.*, 79 (1980) 165–192.