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STRUCTURE OF AN ANTI-COMPLEMENTARY ARABINOGALACTAN FROM THE ROOT OF *Angelica acutiloba* KITAGAWA*

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ABSTRACT

Mild acid hydrolysis of an anti-complementary arabinogalactan (AGIIb-1), isolated from the roots of *Angelica acutiloba* Kitagawa, gave one neutral (N-I) and two acidic arabinogalactan (A-I and A-II) units and one neutral arabinan unit (N-II). The methyl-esterified rhamnogalacturonan cores of A-I and A-II were subjected to base-catalysed β-elimination in the presence of sodium borodeuteride to give the neutral side-chains. Gel filtration showed that A-I contained a high-molecular-weight arabinogalactan and two oligosaccharides consisting of Ara and Gal or Ara as the side chains, and that A-II was composed of two high-molecular-weight arabinogalactans. Similar treatment of the acidic fraction, obtained by partial acid hydrolysis of A-I, gave mono- to tetra-galactosylgalactitol-*1-d*.

Partial acid hydrolysis showed that the rhamnogalacturonan core of A-I contained the sequences →4)-GalA-(1→2)-Rha-(1→ and →4)-GalA-(1→4)-Rha-(1→. Digestion with exo-β-D-galactosidase and Smith degradation indicated that N-I contained a (1→3)-linked galactan backbone with 6-linked galactosyl side-chains. The galactan components of A-I and A-II contained chains that were rich in 3-linked Gal. Fluorescent-labelling with 2-aminopyridine indicated that Ara was the reducing terminal in N-II, whereas it was Rha in A-I and A-II. The mild acid treatment of a different AGIIb-1 preparation gave an oligosaccharide fraction which consisted mainly of terminal and 3-linked Ara*p*, 4- or 5-linked Ara, terminal, 4-linked, and 4,6-linked Gal, and terminal GlcA, and contained Gal and Rha as the reducing terminals.

INTRODUCTION

The anti-complementary arabinogalactan AGIIb-1, isolated[2] from the root of *Angelica acutiloba* Kitagawa (Japanese name, Yamato-Tohki), is a complex pectic arabinogalactan[3] that consists[4] of one neutral (N-I) and two acidic (A-I and A-II) arabinogalactans and one neutral arabinan (N-II), which are linked to each

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other by acid-labile linkages[4]. The mild acid treatment[4] decreases the anti-complementary activity of AGIIb-1. N-I, A-I, and A-II consist[3,4] mainly of 6-linked Gal possessing Ara*f* side-chains at position 3 of Gal, and A-I and A-II each contain[4] a rhamnogalacturonan core with Gal and some neutral side-chains consisting of Ara and Gal. N-II is a (1→5)-α-L-arabinan highly branched at position 3 with Ara*f* side-chains. Digestion with exo-α-L-arabinofuranosidase indicated[4] N-I and N-II to be attached to the rhamnogalacturonan cores of A-I and A-II either directly or indirectly, but details of the total structure of AGIIb-1 were not clarified.

The total structure of AGIIb-1 is now reported.

EXPERIMENTAL

Materials. — The root of *Angelica acutiloba* Kitagawa was purchased from Tochimoto Tenkaidoh Co. AGIIb-1 was prepared[2] from the root by hot-water extraction, precipitations with ethanol and Cetavlon (cetyltrimethylammonium bromide), and chromatography on DEAE-Sephadex, Sephadex G-100, and *Ricinus communis* agglutinin (RCA)-coupled Sepharose. Two different preparations of AGIIb-1 were used. The carbohydrate units N-I, A-I, and A-II were purified from AGIIb-1 as described[4]. DEAE-Sephadex A-25 and A-50, and Sephadex G-100 and G-10 were purchased from Pharmacia, and Bio-gel P-2 (200–400 mesh) and P-4 (−400 mesh) from Bio-Rad. Exo-β-D-galactosidase (jack bean) was obtained from Seikagaku Kogyo.

General. — Total carbohydrate, uronic acid, and pentose in column eluates were assayed by the phenol–sulfuric[5], *m*-hydroxybiphenyl[6], and phloroglucinol–acetic acid[7] methods, respectively. Molecular weights were estimated from the calibration curve of the elution volumes of standard dextrans (T-2000, 500, 70, 40, and 10; Pharmacia) and pullulans (P-50, 20, 10, and 5; Showa Denko Co.) from Sepharose CL-6B and Sephadex G-100 in 0.2M sodium chloride. Samples were hydrolysed with 2M trifluoroacetic acid at 121° for 1 (neutral samples) or 1.5 h (acidic samples), and the hydrolysates were analysed by t.l.c. on cellulose using ethyl acetate–pyridine–acetic acid–water (5:5:1:3). Reducing sugars were detected with alkaline silver nitrate[8] and uronic acids with *p*-anisidine hydrochloride[9]. Sugars were converted conventionally into the alditol acetates. G.l.c. was carried out as described[3]. The molar ratios of alditol and alditol-*1-d* acetates were calculated from the ratios of relative abundance of the MH^+ ions [*m/z* 363 (arabinitol), 377 (rhamnitol), 435 (galactitol), 364 (arabinitol-*1-d*), 378 (rhamnitol-*1-d*), and 436 (galactitol-*1-d*)] in g.l.c.–c.i.-m.s. G.l.c.–c.i. (isobutane)-m.s. was performed with a JEOL DX-300 mass spectrometer equipped with an SPB-1 capillary column (0.25 μm film thickness, 30 m × 0.25 mm i.d., SUPELCO) with the temperature programme 120→220° at 4°/min.

Release of the neutral carbohydrate side-chains from the rhamnogalacturonan cores. — (a) In AGIIb-1, A-I, and A-II. The procedure reported[10] was used. A-I and A-II were methyl-esterified with diazomethane, then heated in 0.1M sodium

hydroxide containing 0.2M sodium borodeuteride at 100° for 3 h. The procedure was repeated five times. Each product was fractionated on DEAE-Sephadex A-25 ($HCOO^-$ form), and neutral (N_I and N_{II}) and acidic (A_I and A_{II}) fractions were obtained by elution with water and 5M HCOOH, respectively. Similar treatment of AGIIb-1 gave a neutral fraction.

(b) In the product of partial acid hydrolysis of A-I. A-I was treated with 0.1M trifluoroacetic acid at 100° for 1 h. The hydrolysates were eluted from DEAE-Sephadex A-25 ($HCOO^-$ form) with water and 5M HCOOH, to give neutral and acidic fractions, respectively. The acidic fraction was reduced with sodium borohydride, desalted with AG50W-X8 (H^+) resin, methyl-esterified, and treated as described above. The neutral carbohydrate side-chains released were isolated by chromatography on DEAE-Sephadex A-25 ($HCOO^-$ form).

Partial acid hydrolysis. — A-I was hydrolysed with 10mM hydrochloric acid at 100° for 1 h. The hydrolysate was eluted from DEAE-Sephadex A-25 ($HCOO^-$ form) with water and 2M HCOOH, to give the neutral and acidic fractions, respectively. The acidic fraction was further treated with 0.1M trifluoroacetic acid at 121° for 1 h, and the second acidic fraction was isolated by the procedure described above. The second acidic fraction was eluted from Sephadex G-25 with 50mM acetate buffer (pH 5.2), to give the oligosaccharide fraction (PA-4) in the region of the lowest molecular weight.

Smith degradation. — N-I, A-I, and A-II were each treated (100 h, 4°) with 90mM sodium metaperiodate in 50mM acetate buffer (pH 4.2). Each reaction was terminated by stirring with ethylene glycol, the products were recovered by dialysis and reduced with sodium borohydride, and the resulting polyalcohols were treated with 0.1M sulfuric acid at room temperature for 24 h. Each solution was neutralised ($BaCO_3$) and desalted with AG50W-X8 (H^+) resin. The product N-I-SD-1 (from N-I) was recovered in the void volume of Bio-gel P-2 and the other products (A-I-SD from A-I, and A-II-SD-1 from A-II) were recovered by lyophilisation. N-I-SD-1 and A-II-SD-1 were each subjected to Smith degradation, and N-I-SD-2 (from N-I-SD-1) was eluted in the void volume of Bio-gel P-4, whereas A-II-SD-2 (from A-II-SD-1) was eluted in the void volume of Sephadex G-10.

Digestion with exo-β-D-galactosidase. — Samples were digested with exo-β-D-galactosidase from jack bean in 50mM acetate buffer (pH 4.0) at 37° for 4 days.

Analysis of the reducing terminal glycosyl residues. — AGIIb-1 (10 mg) was treated[4] with 10mM hydrochloric acid (100°, 10 min), the products were fractionated on Sephadex G-10, and the fraction (MA-AGIIb-1) eluted in the void volume and Ara were obtained. To a solution of MA-AGIIb-1 (8 mg) in water (1 mL) was added 80 μL of the reagent[11] (83 mg of 2-aminopyridine, 32 mg of sodium cyanoborohydride, 37 μL of acetic acid, and 330 μL of methanol), and the solution was heated at 75° for 5 h, then mixed with AG50W-X2 (H^+) resin. The resin was washed with water, and the absorbed fraction was eluted with 0.6M ammonia. Carbohydrate was detected only in the unabsorbed fraction. The carbohydrate units labelled with 2-aminopyridine were purified by chromatography on DEAE-

Sephadex A-50 ($HCOO^-$ form) and Sephadex G-100, followed by h.p.l.c.[12] using a Waters model ALC/GPC 244 equipped with a column (0.75 × 60 cm) of TSK-gel G4000PW (Toyo Soda Co.) and elution with 20mM ammonium acetate buffer (pH 7.5) at 0.5 mL/min. The products were hydrolysed with 2M trifluoroacetic acid at 121° for 1 (neutral units) or 1.5 h (acidic units). The glycosyl residues labelled with 2-aminopyridine (PA-sugars) in the hydrolysates were analysed[12] by h.p.l.c. on a column (0.6 × 15 cm) of YMC-pack A-312 (Yamamura Chemical Laboratory Co.) by elution with 0.25M sodium citrate buffer (pH 4.0) containing 1% of acetonitrile at 0.8 mL/min, and detection using excitation and emission wavelengths of 320 and 400 nm, respectively.

Methylation analysis. — Each sample was methylated (Hakomori[13]), and the product was purified[14] using a Sep-pak C_{18} cartridge (Waters Assoc.). The acidic oligosaccharide fraction (PA-4) was reduced with sodium borodeuteride before methylation, and uronic acids in methylated PA-4 were reduced[14] with sodium borodeuteride in tetrahydrofuran–ethanol (7:3), and then remethylated. The methylated neutral carbohydrate side-chains released from partially hydrolysed A-I were eluted from Sephadex LH-20 with chloroform–methanol (1:1), to give fractions of high (MN-1) and low molecular weight (MN-2). The methylated samples were hydrolysed with 2M trifluoroacetic acid at 121° for 1.5 h, and the products were converted into the partially methylated alditol acetates, and analysed[4] by g.l.c. and g.l.c.–m.s. on a SPB-1 capillary column.

G.l.c.–c.i.- and e.i.-m.s. of methylated oligosaccharide-alditols. — The methods were as described previously[4]. C.i.- and e.i.-mass-spectral fragment ions [A, J, and alditol (ald)][15] were used to determine the structures.

F.a.b.-m.s. of MN-2. — A JEOL DX-300 mass spectrometer was used. A solution of MN-2 in acetone–diethanolamine (1:1) was loaded on a silver plate, and excess of solvent was removed with air. The accelerating voltage was 3 kV, and 3-keV Xe^+ was used as the primary ion.

RESULTS

Release of the neutral carbohydrate side-chains from the rhamnogalacturonan cores in the acidic arabinogalactan units (A-I and A-II) of AGIIb-1. — A-I and A-II were suggested[4] to consist of a rhamnogalacturonan core with neutral carbohydrate side-chains. Base-catalysed β-elimination in the presence of sodium borodeuteride[10] releases the neutral carbohydrate side-chains attached to position 4 of GalA in the rhamnogalacturonan core as glycosylalditol-*1-d* derivatives. Thus, A-I and A-II gave neutral (N_I and N_{II}) and acidic (A_I and A_{II}) fractions (Fig. 1 and Scheme 1). N_I and N_{II} were composed mainly of Ara, Gal, and Rha in the molar ratios 4.9:9.4:1.0 and 5.3:11.0:1.0, respectively, whereas A_I and A_{II} consisted mainly of Ara, Gal, and Rha in the molar ratios 0.6:1.4:1.0 and 0.9:0.9:1.0, respectively, in addition to GalA. Each acidic fraction was subjected to the base-catalysed β-elimination reaction, but no neutral fractions were obtained (data not shown).

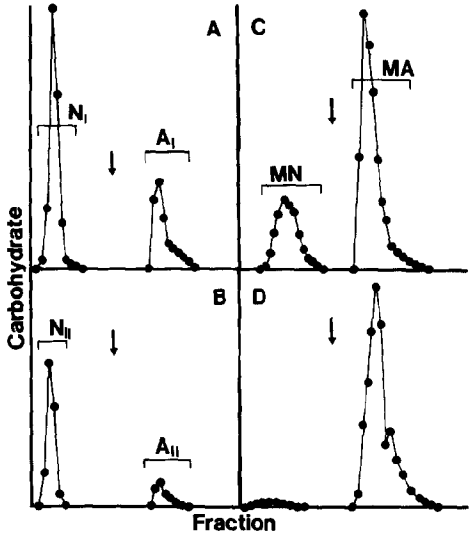
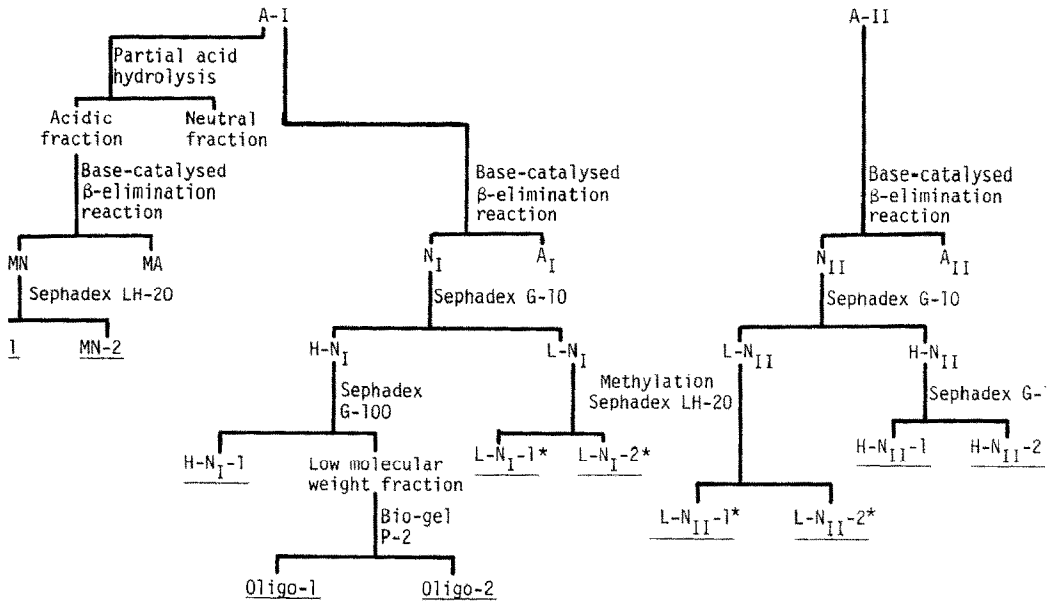


Fig. 1. Anion-exchange chromatography of base catalysed β-elimination product on DEAE-Sephadex: A, A-I; B, A-II; C, the acidic fraction obtained from A-I by partial acid hydrolysis; D, the acidic fraction MA from C (see text). The acidic fractions were eluted with 5M HCOOH at the positions of the arrows.

Analysis of the heterogeneity of N_I and N_{II} (Scheme 1). — N_I and N_{II} were each fractionated on Sephadex G-10 to give components of high (H-N_I and H-N_{II}) and low molecular weight (L-N_I and L-N_{II}) (Fig. 2). H-N_I was eluted from Sephadex G-100 with water to give N-H_I-1 in the void volume, and a fraction of lower molecular weight (Fig. 3A) which was fractionated further on Bio-gel P-2 to give two oligosaccharide fractions (Oligo-1 and -2) (Fig. 3B). Oligo-1 and -2 were each eluted as a single peak from Bio-gel P-4 (data not shown). Elution of H-N_{II} from Sephadex G-100 with water gave a broad peak (Fig. 3C), but elution with 0.2M sodium chloride gave fractions (H-N_{II}-1 and -2) of high molecular weight (Fig. 3D).

When AGIIb-1 was subjected to a β-elimination reaction, the resulting neutral products were eluted from Sephadex G-100 only in the void volume (Fig. 3E).

H-N_I-1, H-N_{II}-1, and H-N_{II}-2 were composed mainly of Ara and Gal in the molar ratios 0.4:1.0, 0.9:1.0, and 0.7:1.0, respectively. Oligo-1 and -2 consisted mainly of Gal and Ara in the molar ratios 1.0:0.4 and 1.0:4.2, respectively. Methylation analysis (Table I) showed that H-N_I-1 contained mainly terminal Ara*p*, and terminal, 3-, 6-, and 3.6-linked Gal, whereas H-N_{II}-1 and -2 consisted mainly of terminal Ara*f*, 4- or 5-linked and 3,4- or 3,5-linked Ara, and 3,6-linked Gal. H-N_{II}-1 also mainly contained 3-linked Gal. Oligo-1 was composed mainly of 6-, 3,6- and 4,6-linked Gal, whereas Oligo-2 consisted mainly of terminal and 3-linked Ara*p*, 4- or 5-linked Ara, and 6-linked Gal. These results suggested that A-I contained one high-molecular-weight galactan possessing some Ara and Gal side-



Scheme 1. Fragmentation of carbohydrate side-chains from the acidic units in AGIIb-1: ⋆ indicates minor fractions.

chains and two oligosaccharide chains attached to the rhamnogalacturonan core through position 4 of GalA. Oligo-1 was suggested to be galacto-oligosaccharide possessing some Ara side-chains, and Oligo-2 to be a branched Ara*p*-rich oligosaccharide chain. A-II contained at least two high-molecular-weight arabinogalactans, which were rich in branched Ara chains. However, the formation of alditol-*1-d* derivatives that would indicate the points of attachment to position 4 of GalA could not be detected in these neutral carbohydrate side-chains from A-I and A-II, because of their low contents.

Analysis of the linkage region between neutral side-chains and GalA in the acidic arabinogalactan units of AGIIb-1. — L-N_I and L-N_{II} (see above) were each methylated, and the products were eluted from Sephadex LH-20 to give fractions of high (L-N_I-1 and L-N_{II}-1) and lower molecular weight (L-N_I-2 and L-N_{II}-2) (data not shown). Methylation analysis (Table II) showed that L-N_I-1 contained 6-, 4,6-, and 3,6-linked Gal, whereas L-N_{II}-1 consisted of terminal Gal and 2,4-linked Rha. L-N_I-1 and L-N_{II}-1 also contained 2-linked Rha-ol-*1-d* and 3-linked Gal-ol-*1-d*. L-N_I-2 was composed of terminal and 2-linked Ara*f*, terminal and 3-linked Ara*p*, and 4- or 5-linked Ara, whereas L-N_{II}-2 consisted of terminal Ara*p* and Gal. L-N_I-2 and L-N_{II}-2 also contained unsubstituted and 3-linked Gal-ol-*1-d*, and 2-linked Rha-ol-*1-d*. These results suggested that the short side-chains in A-I and A-II were attached to position 4 of GalA in the rhamnogalacturonan cores through neutral sugars such as 3-linked Gal (**1**), and terminal Gal was also attached to position 4 of GalA (**2**). The methylated oligosaccharide-alditols in L-N_I-2 and L-N_{II}-2 were each

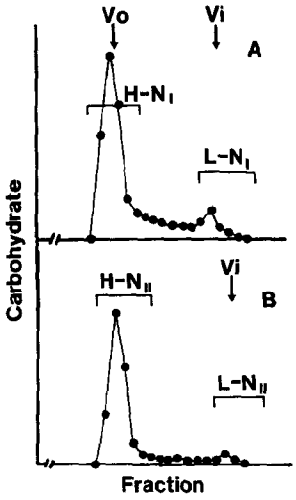


Fig. 2. Gel filtration on Sephadex G-10 of the neutral fractions (N) from Fig. 1: A, A-I; B, A-II.

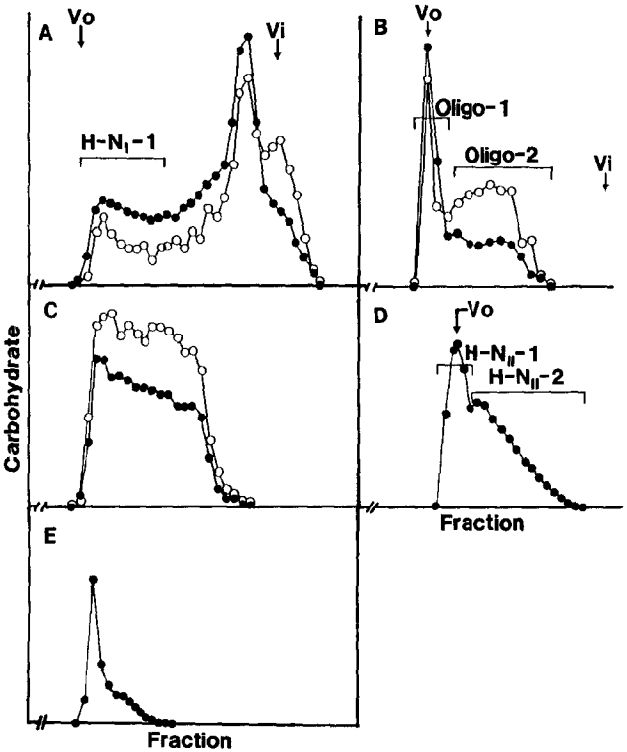


Fig. 3. A, Gel filtration (water) on Sephadex G-100 of H-N$_I$ from Fig. 2A: carbohydrate 490 nm (●), and pentose 552 nm (○); B, elution of the low-molecular-weight fraction from Fig. 2A on Bio-gel P-2; C, gel filtration (water) on Sephadex G-100 of H-N$_{II}$ from Fig. 2B; D, gel filtration (0.2M NaCl) on Sephadex G-100 of H-N$_{II}$ from Fig. 2B; E, gel filtration on Sephadex G-100 of neutral fraction obtained from AGIIb-1 by base-catalysed β-elimination in the presence of sodium borodeuteride.

TABLE I

METHYLATION ANALYSIS OF THE NEUTRAL SIDE-CHAINS OBTAINED FROM A-I AND A-II (SEE SCHEME 1)

Glycosyl residue	*Position of OMe groups*	*Composition (mol. %)*				
		A-I			*A-II*	
		H-N_I-1	*Oligo-1*	*Oligo-2*	*H-N_{II}-1*	*H-N_{II}-2*
Ara	2,3,5	5.4	1.4	4.9	15.5	19.4
	2,3,4	9.0	3.4	21.5	n.d.	n.d.
	2,5	n.d.[a]	1.5	6.4	n.d.	n.d.
	2,3	5.3	3.4	14.7	12.5	14.4
	2,4	n.d.	3.5	11.6	n.d.	n.d.
	2	1.5	1.7	3.5	16.5	20.1
	3	n.d.	0.9	2.6	n.d.	n.d.
Gal	2,3,4,6	11.7	4.1	8.0	7.0	8.1
	2,3,6	4.2	4.6	2.2	3.2	3.6
	2,4,6	11.0	4.6	2.2	10.4	5.8
	2,3,4	22.1	24.0	10.6	8.5	7.8
	2,3	6.2	10.4	6.1	4.9	5.6
	2,4	22.0	28.3	2.9	18.5	13.0
	2	1.6	6.0	3.0	1.8	1.1
Rha	3	0.8	1.8	n.d.	1.2	1.2

[a]Not detected.

analysed by g.l.c.–m.s. (Table III). Only one disaccharide-alditol was detected in L-N_{II}-2, which c.i.-m.s. indicated to have a hexosyl→6-deoxyhexitol-*1-d* unit, and the specific fragment ions (ald in Table III) in e.i.-m.s. suggested it to be Gal-(1→4)-Rha-ol-*1-d*. Since L-N_I and L-N_{II} were minor fractions among the products obtained from A-I and A-II by β-elimination, the linkage regions of high-molecular-weight neutral side-chains were not known. Therefore, the rhamnogalacturonan core with short neutral side-chains was prepared by partial acid hydrolysis of the acidic arabinogalactan units (Scheme 1).

A-I (A-II was not used because the amount was too small) was partially hydrolysed with 0.1M trifluoroacetic acid, and the resulting acidic fraction (data not shown) consisted mainly of Rha, Ara, and Gal in addition to GalA. Methylation analysis indicated 4-linked GalA and 2- and 2,4-linked Rha in addition to Ara and Gal. Therefore, the acidic fraction consists of a rhamnogalacturonan core with short neutral side-chains. The acidic fraction was reduced with sodium borohydride, then subjected to the base-catalysed β-elimination reaction to give neutral (MN) and acidic (MA) fractions (Fig. 1C). Most of MA was recovered as an acidic fraction after the further β-elimination reaction (Fig. 1D). MN consisted mainly of Gal with traces of Ara and Rha, whereas MA contained Rha, Ara, and Gal in addition to GalA. The component sugars of MN were analysed as alditol acetates by g.l.c.–c.i.-m.s., and the molar ratio of hexa-acetates of galactitol and galactitol-*1-d* was calculated to be 0.8:0.2 from the abundances of their protonated molecular

TABLE II

METHYLATION ANALYSIS OF L-N-1 AND -2 OBTAINED FROM A-I AND A-II (SEE SCHEME 1) BY BASE-CATALYSED β-ELIMINATION IN THE PRESENCE OF SODIUM BORODEUTERIDE

Glycosyl residue	*Position of deuterium*	*Position of OMe groups*	*Composition (mol. %)*			
			A-I		*A-II*	
			L-N$_I$-1	*L-N$_I$-2*	*L-N$_{II}$-1*	*L-N$_{II}$-2*
Ara		2,3,5	5.2	34.7	6.8	4.9
		2,3,4	6.8	13.0	5.6	10.5
		3,5	0.3	11.3	n.d.	n.d.
		2,5	1.8	n.d.	n.d.	n.d.
		2,3	6.8	14.4	4.5	n.d.
		2,4	6.3	10.1	4.3	n.d.
		2	2.9	n.d.	5.0	n.d.
Gal	1-*d*	1,2,3,4,5,6	n.d.[a]	trace	n.d.	trace
	1-*d*	1,2,4,5,6	trace	trace	trace	trace
		2,3,4,6	6.7	4.5	16.1	55.3
		2,3,5,6	n.d.	2.9	n.d.	n.d.
		2,3,6	7.5	n.d.	8.7	5.0
	6,6-d_2	2,3,6	6.0	n.d.	8.5	5.5
		2,4,6	3.4	n.d.	2.4	9.4
		2,3,4	23.2	7.6	8.5	n.d.
		2,3	10.2	n.d.	9.6	n.d.
		2,4	11.1	n.d.	5.5	n.d.
		2	2.1	n.d.	n.d.	n.d.
Rha	1-*d*	1,3,4,5	trace	trace	trace	trace
		3	5.3	1.4	12.2	9.4

[a]Not detected.

ions. These results suggested that 20% of the Gal in MN was released from position 4 of GalA by the β-elimination reaction.

MN was methylated (Hakomori), and the product was eluted from Sephadex LH-20 to give fractions of high (MN-1) and low molecular weight MN-2). Methylation analysis (Table IV) showed that MN-1 and -2 consisted mainly of terminal, 4-linked, and 6-linked Gal. However, methylated alditols-*1-d* could not be detected because of their volatilities. F.a.b.-m.s. of MN-2 gave ions [M + DEA (diethanolamine) + H] at *m/z* 576, 577, 780, 781, 984, 985, and 1188 in the ratios 17.3:7.8:10.0:5.0:3.8:2.4:1.0, suggesting MN-2 to contain mono-, di-, and tri-galactosylgalactitol-*1-d* in addition to mono-, di-, tri-, and tetra-galactosyl-galactitols. Methylated MN-2 was also analysed by g.l.c.–c.i.- and e.i.-m.s., and several peaks due to mono- and di-galactosylgalactitols were observed (data not shown). Some peaks showed MH^+ ions at *m/z* 471 and 472 in c.i.-m.s. due to galactosylgalactitol and galactosylgalactitol-*1-d*, respectively, and some peaks showed fragment ions (abJ_2) at *m/z* 439 and 440 due to digalactosylgalactitol and

TABLE III

DIAGNOSTIC FRAGMENT IONS IN C.I.- AND E.I.-M.S. OF METHYLATED DISACCHARIDE-ALDITOLS IN L-N_{II}-2 FROM A-II (SEE SCHEME 1) BY BASE-CATALYSED β-ELIMINATION IN THE PRESENCE OF SODIUM BORODEUTERIDE

Disaccharide-alditol	*C.i.-m.s. fragment ions* [m/z] *(relative abundance)*						
	$(M + H)^+$	$(M + H)^+ - MeOH$	aJ_2	aJ_2OH_2	bA_1	bA_2	
Hexosyl→6-deoxyhexitol-*1-d*		410 (3.9)	206 (12.0)	224 (2.8)	219 (19.1)	187 (100)	
	E.i.-m.s. fragment ions [m/z] *(relative abundance)*						
	aJ_1	aJ_2	bA_1	bA_2	*ald*		
Gal-(1→4)-Rha-ol-*1-d*	266 (1.8)	206 (15.6)	219 (4.7)	187 (100)	134 (9.3)	90 (15.7)	46 (19.4)

TABLE IV

METHYLATION ANALYSIS OF MN-1 AND MN-2 FROM A-I (SEE SCHEME 1) BY BASE-CATALYSED β-ELIMINATION IN THE PRESENCE OF SODIUM BORODEUTERIDE

Glycosyl residue	*Position of OMe groups*	*Linkages*	*Composition (mol. %)*	
			MN-1	*MN-2*
Gal	2,3,4,6	terminal	35.7	43.6
	2,3,6	4	38.2	12.6
	2,4,6	3	6.1	9.2
	2,3,4	6	20.0	17.9
	2,3	4,6	trace	4.8
	2,4	3,6	trace	2.0
Ara	2,3,5	terminal (furanosyl)	n.d.[a]	8.3
	2,3	4 or 5	n.d.	1.6

[a]Not detected.

digalactosylgalactitol-*1-d*, respectively. These results suggested that some galactosyl chains were attached to 4-linked GalA through Gal.

Partial acid hydrolysis of the rhamnogalacturonan core in the acidic arabinogalactan A-I. — A-I was partially hydrolysed with acid in two steps (see Experimental). The resulting acidic oligosaccharide fraction PA-4 consisted of Rha, Gal, GalA, and GlcA. PA-4 was reduced with sodium borodeuteride, methylated, and then carboxyl-reduced with sodium borodeuteride followed by methylation. G.l.c.–m.s. (Table V) revealed two oligoglycosyl-alditol fragments,

TABLE V

DIAGNOSTIC FRAGMENT IONS IN E.I.-M.S. OF METHYLATED DISACCHARIDE-ALDITOLS IN PA-4 OBTAINED FROM A-I BY PARTIAL ACID HYDROLYSIS

Fragment	*E.i.-m.s. fragment ions* [m/z] *(relative abundance)*									*Disaccharide-alditols*
	aJ_1	aJ_2	bA_1	bA_2	*ald*					
a		206 (89.5)	221 (12.1)	189 (42.0)	309 (26.2)	277 (7.3)	134 (35.0)			GlcA-(1→4)-Rha-ol-*1-d*
b	266 (6.5)	206 (28:0)	221 (58.9)		309 (7.0)	134 (13.3)	147 (5.4)	296 (10.7)	308 (35.9)	GalA-(1→2 and 4)-Rha-ol-*1-d*

[a] and **[b]**, each of which gave fragment ions at *m/z* 221 (bA_1) and 206 (aJ_2); **[a]** was eluted faster than **[b]**. The methylated derivative from Glc was eluted faster than the corresponding Gal derivatives from an SPB-1 capillary column; therefore, **[a]** and **[b]** had GlcA→Rha-ol-*1-d* and GalA→Rha-ol-*1-d* units, respectively. The fragment **[a]** gave specific fragment ions (ald in Table V) at *m/z* 309, 277, and 134 consistent with GlcA-(1→4)-Rha-ol-*1-d*. The fragment **[b]** gave specific ions at *m/z* 309, 308, 296, 147, and 134, suggesting the presence of GalA-(1→2)-Rha-ol-*1-d* and GalA-(1→4)-Rha-ol-*1-d*.

Analysis of the neutral arabinogalactan unit N-I. — N-I was partially hydrolysed with 20mM hydrochloric acid at 100° for 1.5 h to remove the arabinosyl side-chains. The hydrolysate was fractionated on Sephadex G-10 to give the galactan portion (MA-N-I) that consisted of Rha, Ara, and Gal in the molar ratios 0.7:1.0:11.1. MA-N-I was digested with exo-β-D-galactosidase, and the products were fractionated on Sephadex G-10 to give Gal and the enzyme-resistant product (Gal-MA-N-I) which consisted of Rha, Ara, and Gal in the molar ratios 0.5:1.0:5.0. Methylation analysis (Table VI) showed that MA-N-I and Gal-MA-N-I consisted of terminal, 3- and 6-, and 3,6-linked Gal, and terminal Ara*f*, and that terminal, 6-, and 3,6-linked Gal in Gal-MA-N-I were reduced by 71.4, 45.6 and 58.1%, respectively. These results suggested that terminal Gal, attached to branched Gal, and 6-linked Gal were removed by the enzymic digestion, and that the 6-linked galactosyl chain was located in the side chains of N-I.

Smith degradation of N-I gave N-I-SD-1 which consisted only of Gal, and its molecular weight was significantly less than that of N-I (Fig. 4). Smith degradation of N-I-SD-1 gave N-I-SD-2, which was eluted also as a broad peak from Sepharose CL-6B (mol. wt., 2.5×10^4–3.2×10^3) (Fig. 4). Methylation analysis (Table VI) showed that N-I-SD-1 comprised terminal, 3- and 6-, and 3,6-linked Gal, whereas N-I-SD-2 was composed of a large amount of 3-linked Gal in addition to terminal

TABLE VI

METHYLATION ANALYSIS OF THE NEUTRAL ARABINOGALACTAN UNIT (N-I) AND THE PRODUCTS FROM N-I

Methylated sugar	*Composition (mol. %)*			*Molar ratio*	
	N-I	*N-I-SD-1*	*N-I-SD-2*	*MA-N-I*	*Gal-MA-N-I*
2,3,5-Me_3-Ara	11.6	n.d.[a]	n.d.	0.7	0.4
2,3-Me_2-Ara	6.1	n.d.	n.d.	n.d.	n.d.
2-Me-Ara	4.9	n.d.	n.d.	n.d.	n.d.
2,3,4,6-Me_4-Gal	4.8	14.9	14.9	1.4	0.4
2,3,6-Me_3-Gal	1.8	n.d.	n.d.	n.d.	n.d.
2,4,6-Me_3-Gal	9.3	38.5	70.2	1.0	1.0
2,3,4-Me_3-Gal	10.4	31.6	trace	5.7	3.1
2,3-Me_2-Gal	18.2	n.d.	n.d.	n.d.	n.d.
2,4-Me_2-Gal	27.1	14.9	14.9	2.7	1.4
2-Me-Gal	5.8	n.d.	n.d.	n.d.	n.d.

[a]Not detected.

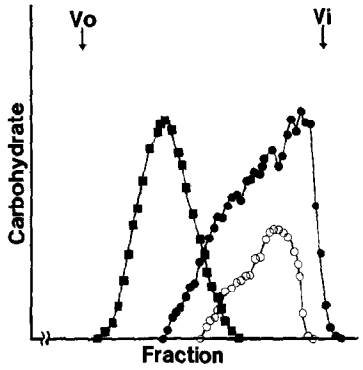


Fig. 4. Gel filtration on Sepharose CL-6B of the Smith-degradation products of N-I: N-I (■), N-I-SD-1 (●), N-I-SD-2 (○).

and 3,6-linked Gal. These results indicated N-I to have a (1→3)-linked galactan backbone.

Analysis of galactan framework in the acidic arabinogalactans A-I and A-II. — Smith degradation of A-I gave A-I-SD, which was composed of Rha, Ara, and Gal in the molar ratios 0.1:0.1:1.0. The mol. wt. of A-I-SD (4.1×10^3) was significantly less than that (17.5×10^4) of A-I. A-I-SD was digested with exo-β-D-galactosidase, then the products were fractionated on Sephadex G-10 to give Gal-A-I-SD-1 in the void volume. The fraction eluted in the inner volume (data not shown) contained Rha and Ara in addition to Gal. Further fractionation on Bio-gel P-2 gave Gal-A-I-SD-2 eluted in the void volume, and Gal (data not shown). GalA-I-SD-1 consisted mainly of Gal, whereas Gal-A-I-SD-2 was composed of Rha, Ara, and Gal in the molar ratios 0.2:0.2:1.0. Gal-A-I-SD-1 and -2 were each eluted as a single peak from Sephadex G-100 (mol. wts. of 5.3×10^3 and 2.4×10^3, respectively). Methylation analysis (Table VII) showed that Gal-A-I-SD-1 contained remarkably increased amounts of 3-linked Gal, whereas Gal-A-I-SD-2 was composed mainly of 2,4-linked Rha, and terminal, 4-, 4,6- and 3,6-, and 3,4,6-linked Gal. These results suggested that A-I contained a 3-linked Gal-rich chain.

Smith degradation of A-II gave A-II-SD-1 which, in turn, gave A-II-SD-2. A-II-SD-1 and -2 were composed of Ara and Gal in the molar ratios 0.9:1.0 and 0.2:1.0, respectively. The mol. wt. of A-II-SD-1 was 3.5×10^3 (*cf.* 17.5×10^4 for A-II), but that of A-II-SD-2 could not be estimated because of the small amount available. Methylation analysis (Table VII) showed that A-II-SD-1 consisted mainly of terminal Ara*f*, and terminal, 3- and 6-, and 4,6-linked Gal, whereas A-II-SD-2 was composed mainly of terminal, 3-, 3,6-, and 3,4,6-linked Gal. These results suggested that A-II also contained a 3-linked Gal-rich chain.

Analysis of the reducing terminals in the carbohydrate units. — AGIIb-1, which was different from the preparation used in the former structural analysis, was treated with 10mM hydrochloric acid at 100° for 10 min to cleave the acid-labile linkages, and the products were fractionated on Sephadex G-10 to give

TABLE VII

METHYLATION ANALYSIS OF A-I AND A-II AND THEIR PRODUCTS

Methylated sugar	*Composition (mol. %)*							
	A-I	*A-I-SD*	*Gal-A-I-SD-1*[a]	*Gal-A-I-SD-2*[a]	*A-II*	*A-II-SD-1*[b]	*A-II-SD-2*[b]	*PA-A-I-3*
2,3,5-Me_3Ara	3.1	6.1	0.9	1.3	3.5	10.9	1.4	4.0
2,3,4-Me_3-Ara	4.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	22.4
2,3-Me_2-Ara	13.2	1.5	1.1	n.d.	9.2	8.2	n.d.	11.0
2,4-Me_2-Ara	6.9	trace	n.d.	n.d.	1.1	n.d.	n.d.	24.9
2-Me-Ara	6.0	trace	1.3	6.1	19.4	6.1	n.d.	8.0
3-Me-Rha	7.4	trace	0.7	11.1	7.0	trace	n.d.	1.6
2,3,4,6-Me_4-Gal	11.1	25.6	11.1	12.3	13.4	26.6	11.8	12.5
2,3,6-Me_3-Gal	14.2	2.6	n.d.	13.6	11.4	n.d.	6.0	13.6
2,4,6-Me_3-Gal	2.3	33.5	53.1	8.8	2.0	10.1	53.0	7.1
2,3,4-Me_3-Gal	21.2	3.8	6.1	8.1	17.5	11.7	n.d.	9.1
2,3-Me_2-Gal	n.d.[c]	7.9	10.8	17.9	n.d.	12.8	n.d.	14.8
2,4-Me_2-Gal	8.6	8.2	14.9	10.4	11.8	7.2	13.5	5.4
2-Me-Gal	1.6	10.7	n.d.	10.4	3.7	6.4	14.3	3.5
2,3,4-Me_3-Glc-*6,6-d_2*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.6

[a]Smith degradation of A-I gave A-I-SD which was digested with exo-β-D-galactosidase. The products were fractionated on Sephadex G-10 to give Gal-A-I-SD-1 in the void volume and a fraction in the inner volume, which gave Gal-A-I-SD-2 in the void volume on Bio-gel P-2. [b]Smith degradation of A-II gave A-II-SD-1, Smith degradation of which gave A-II-SD-2. [c]Not detected.

MA-AGIIb-1 eluted in the void volume and Ara (data not shown). The reducing terminals in MA-AGIIb-1 were labelled[11] with 2-aminopyridine, and the product was fractionated[4] on DEAE-Sephadex A-25 ($HCOO^-$ form) to give neutral (PA-N) and two acidic (PA-A-I and PA-A-II) fractions by elution with water, 2M HCOOH, and 0.2M sodium chloride, respectively (Fig. 5). The fluorescent peak in the neutral fraction was shown to be 2-aminopyridine by h.p.l.c. on TSK-gel G4000PW (data not shown). PA-N was further fractionated on Sephadex G-100 in 0.2M sodium chloride to give a single peak (Fig. 6A). The product in this peak gave mainly Ara on hydrolysis and was probably the neutral arabinan unit (N-II) described previously[4]. Two fluorescent peaks (PA-N-II-1 and PA-N-II-2) were observed in PA-N. H.p.l.c. showed that PA-N-II-2 contained a large amount of 2-aminopyridine in addition to the labelled carbohydrate unit. Fractionation of PA-A-I on Sephadex G-100 gave fractions in the void volume (PA-A-I-1), with intermediate mol. wt., and in the inner volume (PA-A-I-3) (Fig. 6B). PA-A-I-3 also contained a large amount of 2-aminopyridine in addition to the labelled carbohydrate fraction. The acidic arabinogalactan unit, A-I, which was prepared[4] from the other AGIIb-1 preparation, was eluted only in the void volume from Sephadex G-100. PA-A-I-3 consisted of Rha, Ara, and Gal in the molar ratios 0.1:1.2:1.0 in addition to GlcA. Methylation analysis (Table VII) showed that PA-A-I-3 consisted mainly of terminal and 3-linked Ara*p*, 4- or 5-linked Ara, and terminal, 4-, and 4,6-linked Gal, and that GlcA was a non-reducing terminal. PA-A-II was eluted from Sephadex G-100 only in the void volume (Fig. 6C). PA-N-II-1, PA-N-II-2, PA-A-I-1, PA-A-I-3, and PA-A-II were each hydrolysed with 2M trifluoroacetic acid, and the sugars (PA-sugars), which had been labelled with 2-aminopyridine, were analysed[12] by h.p.l.c. on an ODS column. Only PA-Ara was detected in PA-N-II-1 and -2 (Figs. 7B and 7C), and only PA-Rha was detected in PA-A-I-1 and PA-A-II (Figs. 7D and 7F). Thus, PA-N-II-1 and -2 had reducing Ara terminals, and PA-A-I-1 and PA-A-II had reducing Rha terminals. PA-Rha and PA-Gal were detected in PA-A-I-3 (Fig. 7E), indicating it to have reducing Gal and Rha terminals.

DISCUSSION

The acidic arabinogalactan units, A-I and A-II, consisted of one and two high-molecular-weight arabinogalactan side-chains, respectively. Only A-I contained large amounts of neutral chains (Oligo-1 and -2) and both A-I and A-II comprised small amounts of shorter neutral chains (L-N_I-1 and -2, and L-N_{II}-1 and -2). However, AGIIb-1 did not give similar chains by the β-elimination reaction. Therefore, the neutral chains in A-I and A-II were produced by the weak acid treatment of AGIIb-1. Oligo-1 consisted of galacto-oligosaccharide with some Ara side-chains. Since Oligo-2 was obtained as the fraction eluted near Oligo-1 on Bio-gel P-4, Gal in Oligo-2 seemed to be a contaminant from Oligo-1; therefore, Oligo-2 was assumed to be Ara*p*-rich arabino-oligosaccharide. L-N_I-1, L-N_{II}-1, and

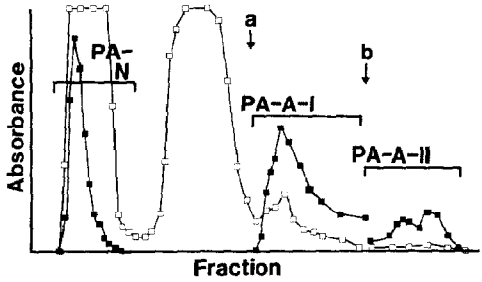


Fig. 5. Anion-exchange chromatography on DEAE-Sephadex A-25 ($HCOO^-$ form) of 2-aminopyridine-labelled products from the weak acid-treated AGIIb-I (MA-AGIIb-1) . The neutral (PA-N) and acidic (PA-A-I and PA-A-II) fractions were obtained by elution with water, 2M HCOOH (a), and 0.2M sodium chloride (b), respectively: carbohydrate, 490 nm (■) and fluorescence (□).

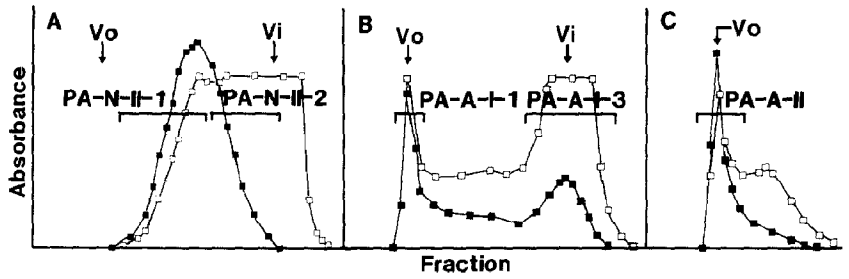


Fig. 6. Gel filtration on Sephadex G-100 of the products from Fig. 5 in 0.2M sodium chloride: A, PA-N; B, PA-A-I; C, PA-A-II. The symbols as in Fig. 5; Vo, void volume; Vi, inner volume.

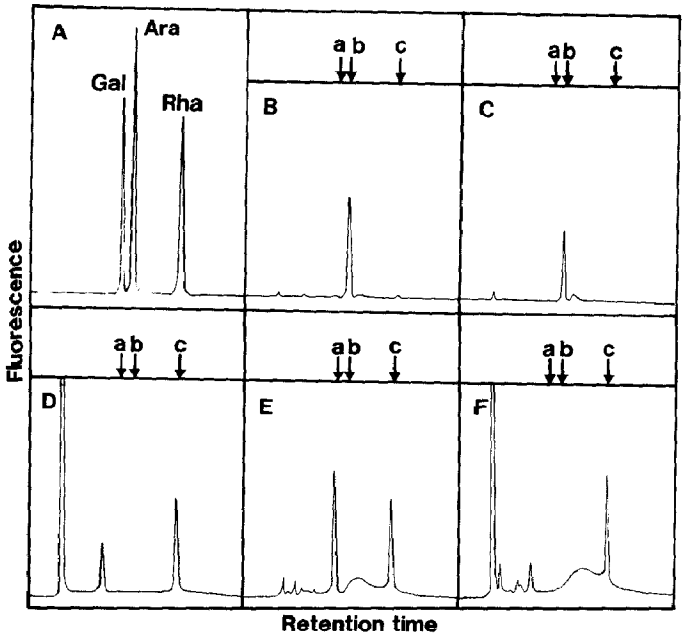


Fig. 7. H.p.l.c. of PA-sugars from the 2-aminopyridine-labelled carbohydrate unit: A, standards; B, PA-N-II-1; C, PA-N-II-2; D, PA-A-I-1; E, PA-A-I-3; and F, PA-A-II; a–c indicate the positions of elution of the standards.

L-N_I-2 might be short galactosyl and arabinosyl chains, respectively.

The present study strongly suggests that the neutral arabinogalactan unit (N-I) consisted of a (1→3)-linked galactan backbone and that (1→6)-linked galactosyl chains, which possessed Ara*f* side-chains at positions 3, were attached to positions 6 of the backbone as the side chains (**3**). A-I and A-II contained 3-linked Gal-rich galactosyl chains derived from high-molecular-weight arabinogalactan side-chains (H-N_I-1, H-N_{II}-1 and -2). Since the galacto-oligosaccharides (MN-1 and MN-2) consisted mainly of 4- and 6-linked Gal (Table IV), the high-molecular-weight arabinogalactan side-chains attached to the rhamnogalacturonan core were assumed to comprise (1→4)- or (1→6)-linked galactosyl chains. Analysis[10] of the linkage region between the neutral side-chains and GalA of another pectic arabinogalactan (AR-4IIc) also showed 4- and 6-linked Gal, in addition to 3-linked Gal, to be attached to position 4 of GalA. Churms and Stephen concluded[16] that an arabinogalactan from *Acacia robusta* contained a galactan backbone in which (1→3)-linked galactosyl chains were attached to other (1→3)-linked galactosyl chains through galactosyl chains that were vulnerable to periodate oxidation. Therefore, the high-molecular-weight arabinogalactan side-chains in A-I and A-II are assumed to contain a backbone consisting of (1→3)-, (1→4)-, and (1→6)-galactosyl chains. The high-molecular-weight arabinogalactan chains (H-N_{II}-1 and -2) in A-II also possessed highly branched arabinosyl side-chains.

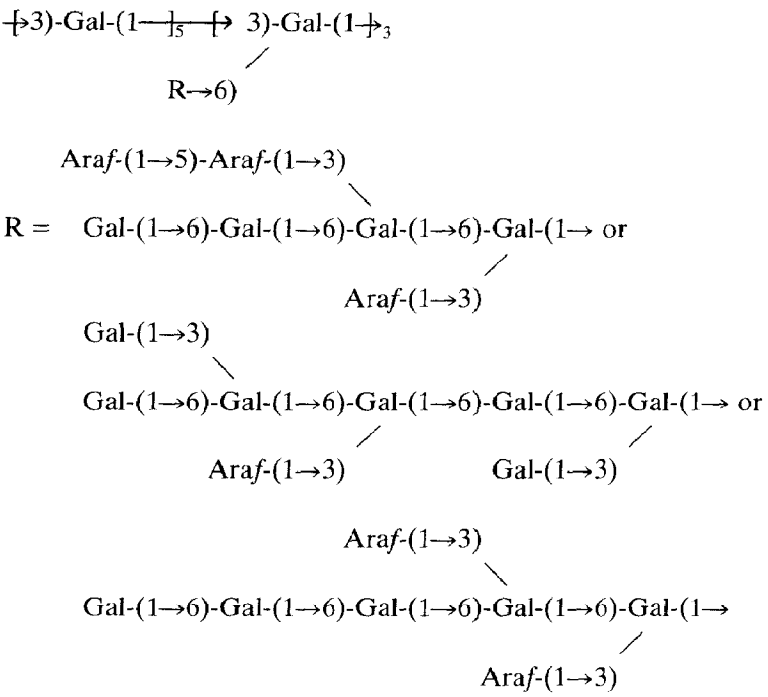
Because A-I and A-II contained the partial structures **1** and **2**, and the galactosyl side-chains possessing galactitol-*1-d* were released from the partially hydrolysed A-I by the β-elimination reaction, the high-molecular-weight arabinogalactan side-chains were attached directly to position 4 of GalA. Structural analysis of the rhamnogalacturonan core in A-I strongly suggested that position 4 of some 2,4-disubstituted Rha in the rhamnogalacturonan core of A-I and A-II was substituted with 4-linked GalA (**4**). Therefore, it is proposed that the high-molecular-weight arabinogalactan and galacto-oligosaccharide chains in A-I and A-II are attached to the rhamnogalacturonan core as shown in **5**. Because Gal-(1→4)-Rha-ol-*1-d* was also detected in fraction L-N_{II}-2, it is also suggested that some Gal might be linked to the rhamnogalacturonan core through position 4 of 2,4-disubstituted Rha in the core (**6**). The linkage region between the arabinosyl chain and GalA was not identified. Arabinosyl chains in the other acidic pectic arabinogalactan (AR-4IIc) from *A. acutiloba* are attached[10] to the rhamnogalacturonan core through position 4 of 2,4-disubstituted Rha; therefore, the arabinosyl chains in A-I were assumed to be attached in the same manner. A similar mode of attachment has been observed in Rhamnogalacturonan I[17] and anti-complementary pectins (AR-2IIa–IId)[1] from *A. acutiloba*.

The carbohydrate units (N-I, N-II, A-I, and A-II) in AGIIb-1 have been proposed[4] to be linked to each other through acid-labile linkages such as the Ara*f* linkage. The present results suggest that N-II, A-I, and A-II are linked to other chains through Ara and Rha, respectively. AGIIb-1, isolated from a different preparation, released only a short galactosyl chain, but not N-I, by treatment with

R-(1→3)-Gal-(1→4)-GalA-(1→ Gal-(1→4)-GalA-(1→

1 **2**

R = galactan or galactosyl chain



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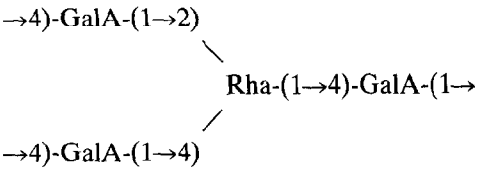
a weak acid, and it is assumed that a neutral high-molecular-weight-arabinogalactan chain such as N-I was present in either A-I or A-II as their neutral side-chains before the treatment with weak acid. The short galactosyl chain possessed reducing Gal or Rha terminals, and N-I might be linked to other chains either through Gal or Rha.

Thus, the total structure of AGIIb-1, illustrated in Fig. 8, can be summarised as follows. (*a*) A-I is probably connected to A-II via Rha in their rhamnogalacturonan cores. (*b*) The neutral arabinan unit (N-II) is probably attached to Ara*p*-rich (Oligo-2) or Ara*f*-rich (L-N_I-2) arabinosyl chains via Ara(*f*). (*c*) High-molecular-weight arabinogalactan side-chains (H-N_I-1), in which 4- or 6-linked Gal-rich portions are attached to the rhamnogalacturonan cores, probably through position 4 of GalA which in turn is attached to position 4 of 2,4-disubstituted Rha in their cores. (*d*) Because the neutral arabinogalactan unit (N-I) contains the (1→3)-linked galactan backbone, N-I is attached to galacto-oligosaccharides



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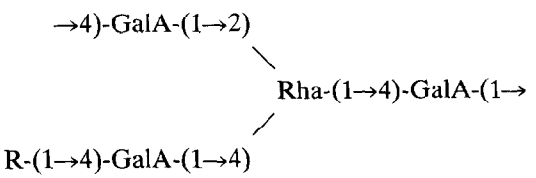

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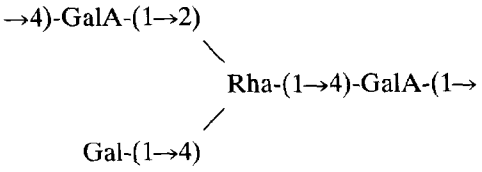

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R = galactan or galactosyl chain

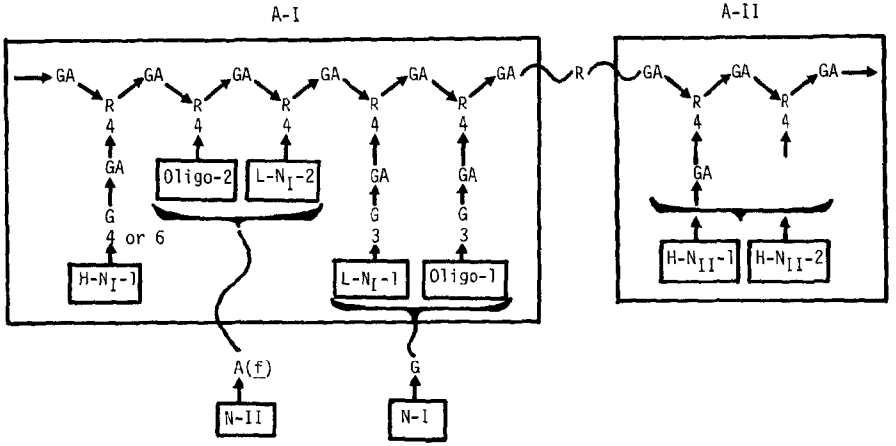

Fig. 8. Proposed total structure of AGIIb-1: GA, 4-linked GalA; R, 2,4-disubstituted Rha; G, Gal; A(*f*), Ara*f*; ~, acid-labile linkages.

(Oligo-1, L-N_I-1, or L-N_I-2) via Gal, and these chains are linked to the rhamnogalacturonan core through 3-linked Gal. (*e*) The linkage regions between the neutral side-chains and the rhamnogalacturonan core in A-II were not identified, but the structures of the linkage regions in A-I and A-II are assumed to be similar. The neutral side-chains (H-N_{II}-1 and -2) may be attached to the rhamnogalacturonan core either through position 4 of 2,4-disubstituted Rha or position 4 of GalA which is attached to position 4 of 2,4-disubstituted Rha in the core.

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