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STRUCTURE OF THE NEUTRAL CARBOHYDRATE SIDE-CHAINS IN ANTI-COMPLEMENTARY ACIDIC POLYSACCHARIDES FROM THE ROOT OF *Angelica acutiloba* KITAGAWA*

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ABSTRACT

The endo-α-(1→4)-polygalacturonase-resistant regions (PG-1a–1c) from three anti-complementary acidic polysaccharides (AR-2IIa–IIc), isolated from the root of *Angelica acutiloba* Kitagawa, were subjected to base-catalysed β-elimination in the presence of sodium borodeuteride. Each PG-1 gave a neutral (*N*) and an acidic fraction (*A*), but each fraction *A* was resistant to further β-elimination. Each fraction *N* consisted of Ara, Gal, and Rha, whereas each fraction *A* contained a large proportion of GalA and small proportions of neutral sugars. Methylation and g.l.c.–m.s. analysis showed that each fraction *N* contained Gal-(1→4)-Rha-ol-*1-d*, Gal-(1→4)-Gal-ol-*1-d*, Gal-(1→6)-Gal-ol-*1-d*, Gal-(1→6)-Gal→Rha-ol-*1-d*, and Gal-(1→6)-Gal-(1→6)-Gal-ol-*1-d*. Methylation analysis suggested that fraction *N*a from AR-2IIa contained a high-molecular-weight galactan with Gal and Ara side-chains, and that fraction *A*a from AR-2IIa consisted mainly of 4-linked GalA together with small amounts of Gal. Degradation of fraction *A*a with lithium in ethylenediamine gave Gal-(1→6)-Gal-ol-*1-d*, Gal→Gal→Gal-ol-*1d*, Gal-(1→6)-Gal-ol, and Gal→Gal→Gal-ol. AR-2IIa and PG-1a reacted with β-glucosyl-Yariv antigen, whereas AR-2IIb–IIc and PG-1b–1c reacted with the antigen weakly or negligibly.

INTRODUCTION

Four pectin-like polysaccharides (AR-2IIa–IId), isolated[1] from the root of *Angelica acutiloba* Kitagawa (Japanese name, Yamato-Tohki), comprise large proportions of polygalacturonan regions and small proportions of "ramified" regions containing the rhamnogalacturonan core and neutral carbohydrate chains that contain[1] mainly Ara and Gal.

Anti-complementary pectic polysaccharides have been isolated from some medicinal plants[2–6] and it is suggested that the neutral carbohydrate side-chains,

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such as arabino-3,6-galactan, contribute to the expression of the activity[3,6] The "ramified" regions from AR-2IIa–IId express similar activities that are higher than those of the original polysaccharides[1], and we now report on the structure of the neutral carbohydrate side-chains.

EXPERIMENTAL

Materials. — The root of *A. acutiloba* Kitagawa was purchased from Tochimoto Tenkaidoh (Japan), and the anti-complementary acidic polysaccharides (AR-2IIa–IId)[1] were isolated by hot-water extraction, precipitations with ethanol and Cetavlon (cetyltrimethylammonium bromide), and anion-exchange chromatography. The α- and β-glucosyl-Yariv antigens were gifts from Dr. A. E. Clarke (Plant Cell Biology Research Center, School of Botany, University of Melbourne). Sephadex G-10, G-50, and LH-20, and DEAE-Sephadex A-25 were purchased from Pharmacia, and Bio-gel P-2 (200–400 mesh) and P-4 (−400 mesh) from Bio-Rad. Pectinase from *Aspergillus niger* was purchased from Sigma, and endo-α-(1→4)-polygalacturonase was purified[7] by Q.-P. Gao in our laboratory.

General. — Total carbohydrate and uronic acid in column eluates were assayed by the phenol–sulphuric acid[8] and *m*-hydroxybiphenyl methods[9], respectively. Samples were hydrolysed with 2M trifluoroacetic acid for 1.5 h at 121°, and t.l.c. of the hydrolysates was performed on cellulose, using ethyl acetate–pyridine–acetic acid–water (5:5:1:3) and detection with alkaline silver nitrate. Neutral sugars and uronic acids were converted[10] into alditol acetates and analysed[3] by g.l.c.

Preparation of "ramified" regions (PG-1a–1c) from AR-2IIa–IIc. — After de-esterification of AR-2IIa–IIc severally in 0.2M sodium hydroxide for 2 h at room temperature, the products were digested with endo-α-(1→4)-polygalacturonase[7] in 50mM acetate buffer (pH 4.2) at 37° for 4 days, then fractionated on Sephadex G-50. The "ramified" regions (PG-1a–1c) were eluted in the void volume.

Gel-diffusion experiments. — AR-2IIa–IId and PG-1a–1c were tested for reactivity to the Yariv antigen by single radial gel-diffusion[11]. β-Glucosyl-Yariv antigen [1,3,5-tri-(4-β-D-glucopyranosyloxyphenylazo)-2,4,6-trihydroxybenzene] was used as the positive Yariv antigen and α-glucosyl-Yariv antigen as the negative antigen. Samples (15 μg) were applied to agarose plates containing Yariv antigen (10 μg/mL) and incubated overnight at room temperature.

Depolymerisation of PG-1a–1c by base-catalysed β-elimination in the presence of sodium borodeuteride. — Each PG-1 (10 mg) was methyl-esterified with diazomethane, then subjected to a base-catalysed β-elimination reaction (0.1M NaOH, 3 h, 100°) in the presence of sodium borodeuteride[12]. The procedure was repeated five times and the final products were fractionated[12] on DEAE-Sephadex A-25 ($HCOO^-$ form) to give neutral (*N*a–*N*c) and acidic fractions (*A*a–*A*c).

Treatment[13] of fraction Aa with lithium in ethylenediamine. — To a solution of fraction *A*a in ethylenediamine was added lithium, the mixture was stirred for 1 h at room temperature and then cooled in ice, and water was added to stop the

reaction. The solvent was evaporated, and the products were reduced with sodium borohydride and desalted with AG50W-X8 (H^+) resin.

Methylation analysis. — Each sample was methylated (Hakomori[14]) and the products were purified[15] using a Sep-pak C_{18} cartridge (Waters Assoc.). The methylated fraction *N*a was eluted from Sephadex LH-20 (chloroform–methanol, 1:1) to give fractions of high (HMW) and low molecular weight (LMW). Uronic acid in the methylated sample was reduced[15] with sodium borodeuteride in tetrahydrofuran–ethanol (7:3). The methylated product was hydrolysed with 2M trifluoroacetic acid for 1.5 h at 121°, and the products were converted into the corresponding alditol acetates and analysed[3] by g.l.c. and g.l.c.–m.s. on an SPB-1 capillary column (SUPELCO).

G.l.c.–m.s. of methylated oligosaccharide-alditols. — The methylated oligosaccharide-alditols obtained from fraction *N*a–*N*c were analysed[16] by g.l.c.–m.s. on an SPB-1 capillary column. C.i.-[17] and e.i.-m.s. fragment ions [A, J, and alditol (ald)][18] were used to determined the structures.

RESULTS

Release of the neutral carbohydrate side-chains from the "ramified" regions of the acidic polysaccharides by base-catalysed β-elimination in the presence of sodium borodeuteride. — The "ramified" regions (PG-1a–1d) from the anti-complementary polysaccharides (AR-2IIa–IId) were each suggested to consist[1] of a rhamnogalacturonan core to which neutral carbohydrate side-chains were attached. The side chains attached to position 4 of GalA residues can be released[12] from the acidic core as alditol-*1-d* derivatives by a base-catalysed β-elimination reaction in the presence of sodium borodeuteride without methyl-etherification of polysaccharides.

Because insufficient PG-1d was available, only PG-1a–1c were studied. The products of each β-elimination reaction were eluted from DEAE-Sephadex A-25 ($HCOO^-$ form), to give a minor neutral fraction (*N*a–*N*c) and a major acidic fraction (*A*a–*A*c) (data not shown). Each fraction *N* comprised Rha, Ara, and Gal (molar ratios of 0.2:0.7:1.0 for *N*a, 0.2:6.1:1.0 for *N*b, and trace:5.2:1.0 for *N*c), whereas each fraction *A* contained small amounts of Rha, Ara, and Gal (molar ratios of trace:0.1:1.0 for *A*a, trace:0.8:1.0 for *A*b, and trace:0.3:1.0 for *A*c) in addition to a large amount of GalA. When fractions *A*a–*A*c were each subjected to β-elimination, most of the material was recovered as an acidic fraction (data not shown).

Analysis of fraction Na. — Fraction *N*a was methylated and the products were separated into fractions of high (HMW) and low molecular weight (LMW) by chromatography on Sephadex LH-20. Methylation analysis (Table I) of HMW gave mainly 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methyl-, 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl-, and 1,3,4,5-tetra-*O*-acetyl-2-*O*-methyl-arabinitol, and 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-, 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-, and 1,3,5,6-tetra-*O*-acetyl-

TABLE I

METHYLATION ANALYSIS OF PRODUCTS (PG-1a, *N*a, HMW, LMW, AND *A*a) DERIVED FROM AR-2IIa

Glycosyl residue	*Deduced glycosidic linkage*	*Composition (mol. %)*				
		PG-1a	N*a*	*HMW*	*LMW*	A*a*
Ara	terminal (furanosyl)	4.0	9.4	4.6	12.1	5.2
	terminal (pyranosyl)	1.7	2.8	0.3	2.0	n.d.
	3- (pyranosyl)	3.2	2.5	1.2	6.8	n.d.
	4- or 5-	3.4	6.8	6.1	10.4	n.d.
	3,4- or 3,5-	1.0	6.4	4.4	4.2	n.d.
Gal	6- (reducing terminal)	n.d.[a]	trace	n.d.	trace	trace
	terminal	4.4	18.9	10.5	22.1	10.8
	4-	3.2	6.0	1.7	7.4	n.d.
	3-	3.4	7.0	5.7	8.5	5.9
	6-	10.7	23.6	47.5	12.5	56.0
	2,4-	6.0	trace	trace	n.d.	2.7
	4,6-	6.5	6.8	2.0	1.5	n.d.
	3,6-	21.3	7.0	11.6	2.0	8.0
	3,4,6-	20.8	trace	1.4	0.4	n.d.
Rha	terminal	1.8	trace	0.6	2.2	n.d.
	2-	1.7	n.d.	trace	trace	n.d.
	2,4-	4.2	n.d.	n.d.	n.d.	n.d.
Glc	terminal	0.3	trace	1.7	2.2	n.d.
Xyl	3,4-	1.8	trace	0.8	1.8	n.d.
GalA	terminal					n.d.
	4-					11.3

[a]Not detected.

2,4-di-*O*-methyl-galactitol, whereas LMW gave mainly 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methyl- and 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl-arabinitol, 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl- and 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-galactitol, and a trace of 6-*O*-acetyl-1,2,3,4,5-penta-*O*-methylgalactitol-*1-d*.

Methylated oligosaccharide-alditols of LMW were analysed by g.l.c.–m.s. (Tables II and III). The methylated disaccharide-alditols **1–3** (Table II) gave fragment ions due to non-reducing termini (bA_1 and bA_2) and alditols (aJ_1 and aJ_2), indicating that **1** had a hexosyl→6-deoxyhexitol-*1-d* unit, and **2** and **3** had hexosyl→hexitol-*1-d* units. The products **1–3** were eluted from an SPB-1 capillary column in this order and they also gave fragment ions due to the ald series. From the results of e.i.–m.s. and the comparison of retention time with those of standard disaccharide-alditols, **1–3** were identified as Gal-(1→4)-Rha-ol-*1-d*, Gal-(1→4)-Gal-ol-*1-d*, and Gal-(1→6)-Gal-ol-*1-d*, respectively. Of the methylated trisaccharide-alditols (**4** and **5**) in LMW (Table III), **4** gave fragment ions at *m/z* 219 and 187 due to the non-reducing terminus (bA_1 and bA_2, respectively), 266 and 206 due to alditol (aJ_1 and aJ_2, respectively), and 391 (bcA_2 produced by elimination of the alditol from the trisaccharide-alditol). Therefore, **4** was considered to be Gal→Gal-→Rha-ol-*1d*. However, the fragment ions assigned to the ald series were not ob-

served, and the glycosidic linkages of the alditol portion could not be deduced. Likewise, the derivative **5** was considered to be Gal→Gal→Gal-ol-*1-d*. Methylation analysis showed that LMW contained a large amount of 6-linked Gal in addition to 6-linked galactitol-*1-d*; therefore, **4** and **5** were assumed to be Gal-(1→6)-Gal→Rha-ol-*1-d* and Gal-(1→6)-Gal-(1→6)-Gal-ol-*1-d*, respectively. Thus, HMW consists mainly of (1→6)-linked galactosyl chains possessing some Gal and Ara side-chains, whereas LMW contained galacto-oligosaccharides with either Rha or Gal at the reducing termini. It was also concluded that PG-1a contained short arabinosyl side-chains consisting of terminal Ara*f* and 4- or 5-linked Ara.

Analysis of fraction A*a*. — Fraction *A*a was eluted as a broad peak from Bio-gel P-4 in 50mM acetate buffer (pH 5.6) (Fig. 1A). Methylation analysis (Table I) showed that fraction *A*a contained mainly Gal and a low proportion of GalA. Since incomplete reduction of GalA in methylated polysaccharides by lithium aluminium hydride has been observed[19], the loss of GalA in the present study is attributed to incomplete reduction of GalA by sodium borodeuteride. It was shown that fraction *A*a contained terminal and 6-linked Gal and 4-linked GalA. Only 6-linked galactitol-*1-d* was formed from *A*a.

TABLE II

DIAGNOSTIC IONS ON E.I.-M.S. OF METHYLATED DISACCHARIDE-ALDITOLS IN FRACTION *N*a

Product	*Fragment ions* [m/z *(relative abundance)*]								*Structure*
	aJ_1	aJ_2	bA_1	bA_2	*ald*				
1	266 (4.1)	206 (51.0)	219 (17.2)	187 (91.7)	319 (0.3)	275 (2.7)	134 (30.2)		Gal-(1→4)-Rha-ol-*1-d*
2	296 (2.5)	236 (27.2)	219 (17.4)	187 (83.4)	362 (5.3)	349 (1.1)	305 (0.4)	134 (21.0)	Gal-(1→4)-Gal-ol-*1-d*
3		236 (20.7)	219 (56.0)	187 (55.3)	337 (0.6)	305 (0.3)	178 (3.8)	146 (20.6)	Gal-(1→6)-Gal-ol-*1-d*

TABLE III

DIAGNOSTIC IONS ON E.I.-M.S. OF METHYLATED TRISACCHARIDE-ALDITOLS IN FRACTION *N*a

Product	*Fragment ions* [m/z *(relative abundance)*]					*Structure*
	aJ_1	aJ_2	bA_1	bA_2	bcA_2	
4	266 (2.7)	206 (30.3)	219 (15.5)	187 (82.4)	391 (1.2)	Gal→Gal→Rha-ol-*1-d*
5	296 (2.6)	236 (6.9)	219 (20.7)	187 (97.9)	391 (1.3)	Gal→Gal→Gal-ol-*1-d*

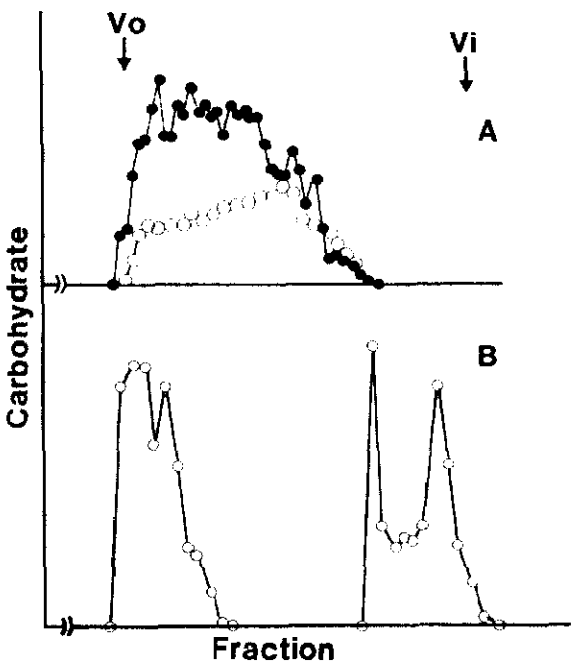

Fig. 1. Gel filtration on Bio-gel P-4 of *A*, fraction *A*a in 50mM acetate buffer (pH 5.6); *B*, lithium-treated fraction *A*a: ○, carbohydrate (490 nm); ●, uronic acid (520 nm); Vo, void volume; Vi, included volume.

Treatment of fraction *A*a with lithium in ethylenediamine[13] decomposed the GalA. Elution of the products from Bio-gel P-4 gave Li-A-1 in the void volume, and lower oligosaccharides Li-A-2 (Fig. 1B). Li-A-1 consisted mainly of Gal, but further analysis could not be performed because of the small amount available. Li-A-2 was methylated (Hakomori[14]), and the products were analysed by g.l.c.–m.s. C.i.-m.s. (Table IV) gave two protonated molecular ions at *m/z* 442 and 441, and fragment ions due to aJ_1 (*m/z* 266 and 265), aJ_2 (*m/z* 206 and 205), aJ_2OH_2 (*m/z* 224 and 223), and bA_1 and bA_2 (*m/z* 219 and 187). These results suggested the presence of a hexosyl→6-deoxyhexitol-*1-d* (**6**) and a hexosyl→6-deoxyhexitol (**7**). In e.i.-m.s. (Table V), **6** gave fragment ions of the ald series at *m/z* 395, 319, 307, and 134, and **6** was assigned as Gal-(1→4)-Rha-ol-*1-d*, whereas **7** gave fragment ions at *m/z* 395, 305, and 293, and was assigned as Gal-(1→2)-Rha-ol. E.i.-m.s. also revealed products **8**–**10**, which were not detected in c.i.-m.s. because of their small amounts. Product **8** gave fragment ions due to aJ_2 at *m/z* 236 and 235, and to bA_1 and bA_2 at *m/z* 219 and 187, suggesting the presence of the disaccharide-alditols **8a** and **8b**. In e.i.-m.s., **8a** gave fragment ions due to ald series at *m/z* 337, 178, 146, and 134, whereas **8b** gave fragment ions at *m/z* 337, 177, 145, and 133, indicating the former to be Gal-(1→6)-Gal-ol-*1-d* and the latter to be Gal-(1→6)-Gal-ol. Product **9** was eluted in the region for a hexosyltrisaccharide-alditol in g.l.c. on an SPB-1 capillary column, and gave fragment ions due to abJ_2 at *m/z* 439, and to aJ_2 and bA_1 at *m/z* 235 and 219, suggesting the structure Gal→Gal→Gal-ol. However, the linkage of the alditol portion could not be deduced because the fragment ions due to ald were not observed. Product **10** was also eluted in the region for a hexosyltrisaccharide-alditol and gave the fragment ions due to abJ_2 (*m/z* 440), bA_1 (*m/z* 219), and aJ_2 (*m/z* 236), indicating the structure Gal→Gal→Gal-ol-*1-d*.

TABLE IV

DIAGNOSTIC IONS ON C.I.-M.S. OF METHYLATED OLIGOSACCHARIDE-ALDITOLS IN Li-A-2 DERIVED FROM FRACTION *A*a BY LITHIUM-MEDIATED DEGRADATION

Product	*Fragment ions* [m/z *(relative abundance)*]							*Oligosaccharide-alditol*
	$(M+H)^+$	$(M+H)^+ -MeOH$	aJ_1	aJ_2	aJ_2OH_2	bA_1	bA_2	
6	442 (15.9)	410 (74.6)	266 (7.3)	206 (12.1)	224 (56.6)	219 (81.3)	187 (100)	hexosyl→ 6-deoxyhexitol-*1-d*
7	441 (6.9)	409 (29.4)	265 (3.5)	205 (6.7)	223 (23.9)	219 (81.3)	187 (100)	hexosyl→ 6-deoxyhexitol

TABLE V

DIAGNOSTIC IONS ON E.I.-M.S. OF METHYLATED OLIGOSACCHARIDE-ALDITOLS IN Li-A-2 DERIVED FROM FRACTION Aa BY LITHIUM-MEDIATED DEGRADATION

Product	*Fragment ions* [m/z *(relative abundance)*]								*Structure*
	aJ_1	aJ_2	bA_1	bA_2	*ald*				
6	266 (8.3)	206 (5.4)	219 (29.8)	187 (100)	395 (0.2)	319 (0.3)	307 (0.7)	134 (25.6)	Gal-(1→4)-Rha-ol-*1-d*
7	265 (4.8)	205 (3.3)	219 (29.8)	187 (100)	395 (0.2)	305 (1.2)	293 (0.7)		Gal-(1→2)-Rha-ol
8a		236 (26.0)	219 (23.2)	187 (91.3)	337 (4.0)	178 (3.1)	146 (18.2)	134 (6.7)	Gal-(1→6)-Gal-ol-*1-d*
8b		235 (56.7)	219 (23.2)	187 (91.3)	337 (4.0)	177 (11.9)	145 (67.8)	133 (60.4)	Gal-(1→6)-Gal-ol
	aJ_2	abJ_2	bA_1	bA_2	bcA_1	bcA_2			
9	235 (56.9)	439 (12.5)	219 (21.1)	187 (100)					Gal→Gal→Gal-ol
10	236 (36.2)	440 (2.0)	219 (10.4)	187 (100)					Gal→Gal→Gal-ol-*1-d*

Analysis of fractions Nb, Nc, A*b, and* A*c.* — Fractions *N*b and *N*c were also analysed as described above. However, the methylated products were not fractionated on Sephadex LH-20 because the amounts available were too small.

When each methylated fraction *N* was subjected to g.l.c.–m.s., the same di- and tri-saccharide-alditols were detected as in fraction *N*a (Tables II and III). Methylation analysis (Table VI) showed that *N*b and *N*c contained mainly terminal Ara*f*, 4- or 5-linked and 3,4- or 3,5-disubstituted Ara, and fraction *N*b also contained

TABLE VI

METHYLATION ANALYSIS OF PG-1b, PG-1c, AND FRACTIONS *N*b, *N*c, *A*b, AND *A*c

Glycosyl residue	*Deduced glycosidic linkage*	*Composition (mol. %)*					
		AR-2IIb			*AR-2IIc*		
		PG-1b	N*b*	A*b*	*PG-1c*	N*c*	A*c*
Ara	terminal (furanosyl)	6.3	11.1	3.8	8.5	25.5	3.5
	terminal (pyranosyl)	2.4	0.7	n.d.	2.7	n.d.	n.d.
	3- (pyranosyl)	9.4	n.d.	n.d.	13.4	n.d.	n.d.
	4- or 5-	9.9	26.1	3.3	12.4	28.9	n.d.
	3,4- or 3,5-	3.3	26.8	n.d.	6.1	25.5	n.d.
Gal	6- (reducing terminal)	n.d.[a]	trace	n.d.	n.d.	trace	n.d.
	terminal	8.8	6.7	9.0	10.6	4.0	3.7
	4-	3.9	12.6	3.7	4.8	8.3	3.9
	3-	3.9	2.6	n.d.	3.4	1.3	10.7
	6-	6.3	8.9	40.5	3.5	5.2	9.5
	2,4-	10.7	n.d.	n.d.	7.3	trace	4.1
	4,6-	4.3	n.d.	n.d.	3.2	trace	n.d.
	3,6-	8.9	4.6	5.6	4.3	1.3	6.4
	3,4,6-	2.8	n.d.	n.d.	1.2	trace	n.d.
Rha	terminal	4.5	n.d.	n.d.	4.4	n.d.	n.d.
	2-	4.1	n.d.	n.d.	3.4	n.d.	n.d.
	2,4-	6.6	n.d.	n.d.	7.8	n.d.	n.d.
Glc[b]	terminal	1.0	n.d.	n.d.	1.2	trace	n.d.
Xyl[b]	3,4-	3.0	n.d.	n.d.	2.0	trace	n.d.
GalA	terminal			n.d.			15.0
	4-			34.0			43.2

[a]Not detected. [b]Not detected in the original polysaccharides.

mainly 4-linked Gal. A trace of 6-*O*-acetyl-1,2,3,4,5-penta-*O*-methylgalactitol-*1-d* was detected. However, some glycosyl residues, such as terminal and 3-linked Ara*p* and various types of Gal, were decreased or lost in each fraction *N* by comparison with each PG-1 of AR-2IIb and IIc, possibly by a "peeling" reaction during the β-elimination reaction.

Methylation analysis of the fractions *A*b and *A*c indicated (Table VI) that fraction *A*b contained a large proportion of 4-linked GalA together with terminal and 6-linked Gal, and that fraction *A*c contained a large proportion of terminal and 4-linked GalA together with 3- and 6-linked Gal.

Analysis of the (1→3,6)-galactan moiety by using the Yariv antigen. — The β-glucosyl-Yariv antigen reacts[20] with (1→3,6)-β-galactan to form a red dye, and (1→3,6)-β-galactan can be quantified[11] by single radial gel-diffusion with the β-glucosyl-Yariv antigen. When AR-2IIa–IId were tested by single radial gel-diffusion with the β-glucosyl-Yariv antigen (Fig. 2), AR-2IIa reacted slightly, whereas AR-2IIb–IId did not react. PG-1a reacted strongly with the antigen, whereas PG-1b reacted slightly, and PG-1c did not react. Each polysaccharide and PG-1 did not

react with α-glucosyl-Yariv antigen as the negative control[20] (data not shown). These results suggested that AR-2IIa contained a large proportion of a (1→3,6)-β-galactan moiety, whereas AR-2IIb–IId contained small or negligible proportions.

DISCUSSION

Acidic pectic polysaccharides[21,22] are considered to contain a polygalacturonan region and a rhamnogalacturonan as the acidic core with neutral carbohydrate chains attached to position 4 of 2,4-disubstituted Rha in the rhamnogalacturonan core to form the "ramified" region. However, the details of structure of the neutral carbohydrate side-chains and their mode of attachment have not yet been clarified.

The present results suggested that the short galactosyl oligosaccharide chains in the neutral carbohydrate side-chains released from the "ramified" regions of AR-2IIa by the β-elimination reaction are attached to position 4 of GalA residues through 4-linked Rha, and 4- and 6-linked Gal as shown in Table VII. Some pectic polysaccharides contain[4,16] the sequence →4)-GalA-(1→4)-Rha-(1→, and it is suggested that short galactosyl chains in AR-2IIa–IIc might be linked to such a chain either as $(Gal)_n$→Gal-(1→4)-GalA-(1→4)-[→2)-Rha-(1→] or $(Gal)_n$→Gal-(1→4)-[→2)-Rha-(1→]. AR-2IIa also contained long galactosyl chains possessing some Gal and Ara side-chains which were assumed to be attached to rhamnogalacturonan as described above. The long galactosyl chains in AR-2IIa only were also assumed to possess a (1→3,6)-galactan moiety because AR-2IIa reacted with the β-glucosyl-Yariv antigen. The result of methylation analysis of LMW suggested that AR-2IIa also contained arabino-oligosaccharide chains. It was also assumed that the "ramified" regions of AR-2IIb and IIc might contain more arabinose-rich side-chains than that of AR-2IIa.

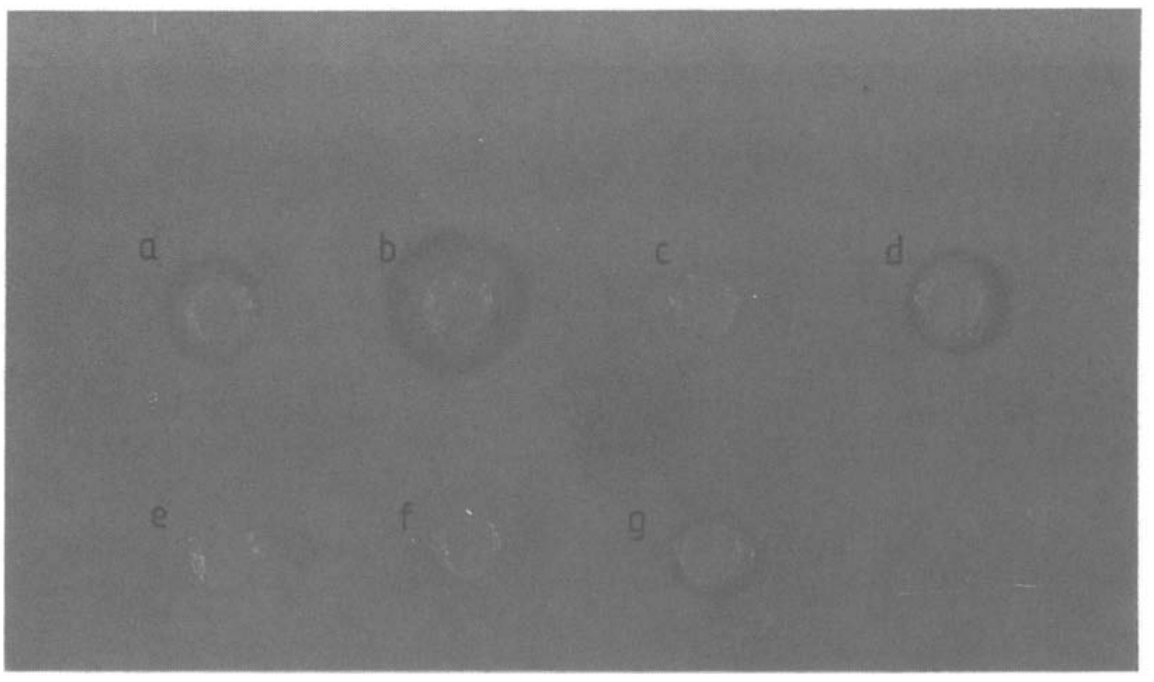

Fig. 2. Single radial gel-diffusion with Yariv antigen: (*a*) AR-2IIa, (*b*) PG-1a, (*c*) AR-2IIb, (*d*) PG-1b, (*e*) AR-2IIc, (*f*) PG-1c, (*g*) AR-2IId.

TABLE VII

PARTIAL STRUCTURES IN AR-2IIa–IIc

Gal-(1→4)-Rha-(1→4)-GalA-(1→
Gal-(1→4)-Gal-(1→4)-GalA-(1→
Gal-(1→6)-Gal-(1→4)-GalA-(1→
Gal→Gal→Rha-(1→4)-GalA-(1→
Gal→Gal→Gal-(1→4)-GalA-(1→

Recently, Lau *et al.* reported[23] that Rhamnogalacturonan I (RG-I) contained various galacto- and arabino-oligosaccharide side-chains similar to those in AR-2IIa.

The base-catalysed β-elimination reaction of the "ramified" regions from AR-2IIa–IIc gave a large proportion of an acidic fraction (*A*a–*A*c), that contained galacto-oligosaccharides. Because some of the galacto-oligosaccharides possessed galactitol at the reducing termini, fraction *A* was produced by incomplete β-elimination of GalA. However, since some galacto-oligosaccharides possessing galactitol-*1-d* were also detected in the products from fraction *A*a after the degradative reaction using lithium, it is suggested that they were attached originally to GalA as in →4)-GalA-(1→$(Gal)_n$-(1→4)-GalA-(1→.

Although the structures of the neutral side-chains in the "ramified" region of AR-2IIa were different from those of AR-2IIb and IIc, each "ramified" region showed[1] similar anti-complementary activity. A pectin[24] from *Zyziphus jujuba* was shown to have[25] no anti-complementary activity, and to contain[24] mainly 4-linked Gal as the neutral sugar. Therefore, the neutral carbohydrate chains possessing the common structure of AR-2IIa–IIc might play a role in the expression of anti-complementary activities of their "ramified" regions. The relationship between the neutral carbohydrate side-chains and anti-complementary activity of "ramified" regions in these polysaccharides is being studied further.

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