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FURTHER STRUCTURAL STUDIES OF ANTI-COMPLEMENTARY ACIDIC HETEROGLYCANS FROM THE LEAVES OF *Panax ginseng* C. A. MEYER

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ABSTRACT

The three potent anti-complementary polysaccharides, GL-PI, GL-PII, and GL-PIV, isolated from the leaves of *Panax ginseng* C. A. Meyer, were subjected to base-catalysed β-elimination in the presence of sodium borodeuteride or enzymic digestion with endo-α-D-(1→4)-polygalacturonase. β-Eliminative degradation of GL-PI and GL-PII each gave neutral (IN and IIN) and acidic (IA and IIA) fractions. Each fraction N consisted of Ara, Rha, Gal, and Glc, whereas each fraction A comprised a large proportion of GalA in addition to Rha, Gal, Glc, and GlcA. Methylation analysis and g.l.c.–m.s. showed that each fraction IN and IIN contained Rha-(1→2)-Rha-ol-*1-d*, Rha-(1→4)-Rha-ol-*1-d*, Ara-(1→4)-Rha-ol-*1-d*, Gal-(1→4)-Rha-ol-*1-d*, Gal-(1→6)-Gal-ol-*1-d*, and GlcA-(1→4)-Rha-ol-*1-d*, and that IA and IIA contained Rha→Rha-ol-*1-d*, HexA→Rha-ol-*1-d*, and HexA→Rha→Rha-ol-*1-d*. Methylation analysis indicated that IN and IIN also contained high-molecular-weight 6-linked galactan and 4-linked glucan, and that IA and IIA consisted mainly of 2-linked Rha, 4-linked GalA, and terminal and 6-linked Gal. IIA contained more 2-linked Rha than IA.

Endo-α-D-(1→4)-polygalacturonase-mediated digestion of GL-PIV produced a high-molecular-weight fraction (PG-1) which was rich in neutral sugars, fragments of intermediate size (PG-2), and oligosaccharides (PG-3). PG-1 contained a rhamnogalacturonan core, galactan (which mainly comprised terminal, 6-linked, and 4,6-disubstituted Gal), and 4-linked glucans. PG-2 contained (1→4)-linked α-galacturonan partially branched at position 2 or 3 and a rhamnogalacturonan core in addition to small proportions of Gal and Glc. PG-3 contained large proportions of oligogalacturonides.

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INTRODUCTION

Four heteroglycans (GL-PI–PIV) have been purified[1] from the leaves of *P. ginseng*. GL-PI, GL-PII, and GL-PIV showed[1] high anti-complementary activities, whereas GL-PIII had low activity. GL-PI, GL-PII, and GL-PIV each appear[1] to consist of a rhamnogalacturonan backbone with side chains mostly at position 4 of 2-linked Rha, whereas GL-PIII consists of a core of rhamnogalacturonan and 2,4- or 3,4-branched GalA with side chains attached mostly at position 2 or 3 of 4-linked GalA.

Several anti-complementary pectic polysaccharides have been purified from Chinese herbs[2–5] and the side chains may be involved in the expression of the anti-complementary activity[6,7].

We now report further on the structures of the neutral side-chains in GL-PI, GL-PII, and GL-PIV.

EXPERIMENTAL

Materials. — The leaves of *P. ginseng* C. A. Meyer, which were cultivated in Jilin, were collected on Chang Bai mountain (China). The fraction (GL-3) of highest anti-complementary activity was prepared by hot-water extraction, and precipitations with ethanol and Cetavlon (cetyltrimethylammonium bromide). GL-3 was fractionated further on DEAE-Sephadex A-50, DEAE-TOYOPEARL 650C, and Sepharose CL-6B to give[1] GL-PI, GL-PII, and GL-PIV. DEAE-Sephadex A-25 and Sephadex G-50 and LH-20 were purchased from Pharmacia, and Bio-Gel P-6 (−400 mesh), P-4 (−400 mesh), and P-2 (200–400 mesh) from Bio-Rad. Pectinase from *Aspergillus niger* was purchased from Sigma, and endo-α-(1→4)-polygalacturonase was purified by the method of Thibault and Mercier[8].

General methods. — Carbohydrate, uronic acid, and 4,5-unsaturated uronic acid were assayed using phenol–sulfuric acid[9], *m*-hydroxylbiphenyl[10], and λ_{max} at 235 nm[11], respectively. Each sample was hydrolysed with 2M trifluoroacetic acid for 1.5 h at 121°, and t.l.c. of each hydrolysate was performed on cellulose, using ethyl acetate–pyridine–acetic acid–water (5:5:1:3). Reducing sugars and uronic acids were detected with alkaline silver nitrate[12] and *p*-anisidine hydrochloride[13]. Neutral sugars and uronic acids were converted into the corresponding alditol acetates[14] and analysed by g.l.c. G.l.c. was carried out at 190° using a Shimadzu GC-6A gas chromatograph equipped with a flame-ionisation detector and a glass column (3 mm i.d. × 200 cm) packed with 1% of OV-225 on Uniport HP. The molar ratios were calculated from the peak areas and molecular weights of the corresponding alditol acetates.

Fragmentation of the acidic moiety in GL-PI and -PII. — GL-PI (20 mg) and GL-PII (25 mg) were each esterified with diazomethane, then subjected to base-catalysed β-elimination in the presence of sodium borodeuteride[15]. The procedure was repeated 7 times. The final products were fractionated on DEAE-Sephadex

A-25 ($HCOO^-$ form), and the neutral [IN (2.1 mg) and IIN (2.8 mg)] and acidic [IA (10.5 mg) and IIA (9.2 mg)] fractions were obtained by elution with water and 5M HCOOH, respectively.

Enzymic digestion of GL-PIV. — To a solution of GL-PIV (6.4 mg) in 20mM acetate buffer (pH 4.2, 3 mL) was added endo-α-(1→4)-polygalacturonase (350 nkat) from *A. niger*. After incubation at 30° for 2 days, the mixture was neutralised with aq. 0.5% NaOH, boiled for 5 min, then fractionated on a column (1.9 × 95 cm) of Sephadex G-50 to give PG-1–3.

Carboxyl-reduction of PG-3. — PG-3 (1.5 mg) was reduced with 1-ethyl-3-(3-dimethylaminopropyl)carbodi-imide (EDC) and sodium borodeuteride in deuterium oxide according to the modified procedure[16] of Taylor and Conrad[17]. The product was desalted with AG50W-X8 (H^+) resin, applied to a column (1 × 45 cm) of Bio-gel P-2 equilibrated with 0.2M acetate buffer (pH 5.6), and eluted with the buffer to give fractions of high (PG-3H) and low (PG-3L) molecular weight.

Methylation analysis. — Each dried sample was methylated once by the method of Hakomori[18] in order to prevent β-elimination, and the completeness of formation of alkoxide was checked by using triphenylmethane[19]. The methylated product was purified[20] using a Sep-pak C_{18} cartridge (Waters Assoc.).

(*a*) Methylated IN and IIN were each fractionated on a column (1 × 25 cm) of Sephadex LH-20, using chloroform–methanol (1:1). The fractions of high and low molecular weight were assayed with the 1-naphthol–sulfuric acid reagent[21].

(*b*) The methylated fractions containing uronic acid were carboxyl-reduced[20] with sodium borodeuteride in tetrahydrofuran–aq. 95% ethanol (7:3) for 18 h at room temperature followed by incubation for 1 h at 75°. The products were desalted with AG50W-X8 (H^+) resin, then remethylated, and the methylated oligoglycosyl-alditols were fractionated on Sephadex LH-20, as described above, to give the fractions of high and low molecular weight.

The methylated products were hydrolysed with 2M trifluoroacetic acid for 1.5 h at 121°, and the products were reduced by sodium borohydride in aq. 95% ethanol containing M ammonium hydroxide for 3 h at room temperature, then converted into the partially methylated alditol acetates, and analysed[22] by g.l.c. and g.l.c.–m.s. on a SPB-1 capillary column (0.25-μm film thickness, 30 m × 0.25 mm i.d., SUPELCO).

G.l.c.–m.s. of methylated oligoglycosylalditols. — G.l.c.–m.s. involved a SPB-1 capillary column (splitless injection and a temperature programme of 180°→310° at 2–4°/min) and a JEOL DX-300 mass spectrometer [e.i. at 70 eV with an ionisation current of 300 μA, and c.i. (isobutane) at 250 eV and an accelerating voltag of 3kV]. C.i.[23] and e.i. fragment ions [A, J, and alditol (*ald*)][24] were used to determine the structures of the methylated oligoglycosylalditols.

High-resolution e.i.-m.s. (70 eV) was performed with a JEOL DX-303 mass spectrometer equipped with a DA-5000 computer system. Samples were separated on a DB-1 megabore column (15 m, J and W Scientific Inc.).

RESULTS

Base-catalysed β-elimination of GL-PI and GL-PII. — Previous results indicated[1] that GL-PI and -PII contained a large rhamnogalacturonan moiety, but little polygalacturonan; therefore, methyl-esterified GL-PI and GL-PII were subjected to base-catalysed β-elimination in the presence of sodium borodeuteride[15]. Each product was fractionated on DEAE-Sephadex A-25 to give neutral (IN from GL-PI and IIN from GL-PII) and acidic (IA from GL-PI and IIA from GL-PII) fractions (Fig. 1). IN and IIN consisted of Ara, Rha, Gal, and Glc in the molar ratios 0.1:0.2:1.0:0.3 and trace:0.1:1.0:0.3, respectively. IA and IIA contained Rha, Gal, and Glc in the molar ratios 1.0:0.3:0.2 and 1.0:0.4:0.2, in addition to GalA and GlcA in the molar ratios 2.0:1.0 and 2.1:1.0, respectively. When each acidic fraction was subjected to base-catalysed β-elimination, no neutral fractions were obtained (data not shown). Fractions IA and IIA were each eluted from a column of Bio-gel P-4 with 0.2M sodium acetate buffer (pH 5.6) (Fig. 2). The main part of the carbohydrate was eluted in the void volume and the 4,5-unsaturated uronic acid (λ_{max} 235 nm) between the void to the included volumes (Fig. 2). Fractions IA1–2 and IIA1–2 were obtained from IA and IIA, respectively. Fractions IA1 and IIA1

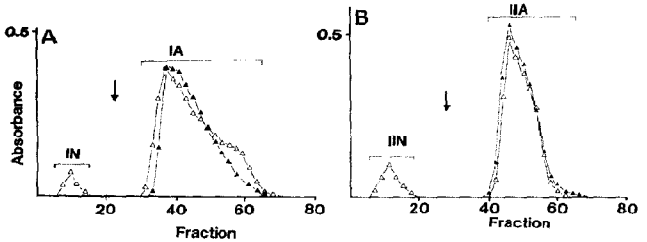


Fig. 1. Chromatography on DEAE-Sephadex of the products obtained from methyl-esterified GL-PI (A) and GL-PII (B) by base catalysed β-elimination. The acidic fractions were eluted with 5M HCOOH at the position of the arrow: △, carbohydrate (490 nm); ▲, uronic acid (520 nm).

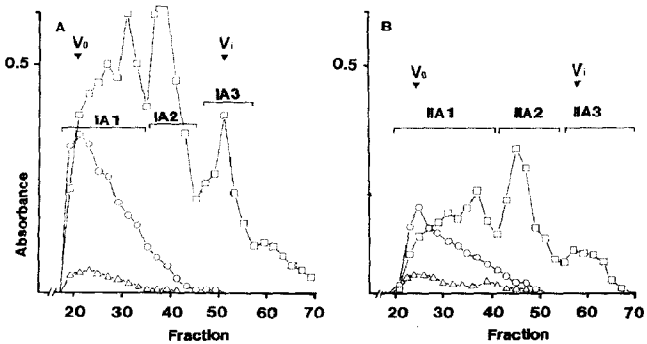


Fig. 2. Gel filtration of the acidic fractions IA and IIA from Fig. 1 on Bio-gel P-4: ○, carbohydrate (490 nm); △, uronic acid (520 nm); □, 235 nm; Vo, void volume; Vi, included volume.

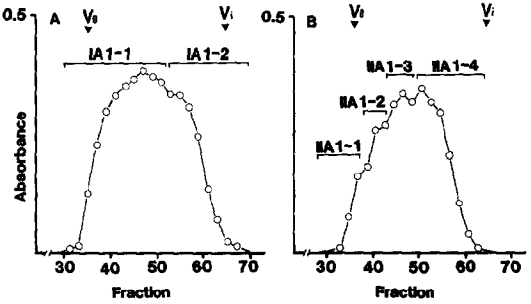


Fig. 3. Gel filtration of IA1 and IIA1 from Fig. 2 on Bio-gel P-6: O, carbohydrate (490 nm).

were each fractionated on Bio-gel P-6 to give IA1-1–2 and IIA1-1–4, respectively (Fig. 3). IA1-1–2 and IIA1-1–4 consisted of Rha, Gal, and Glc in the molar ratios 4.2:1.0:trace, 3.9:1.0:0.4, 2.0:1.0:trace, 3.4:1.0:nil, 1.3:1.0:0.3, and 2.2:1.0:0.4, respectively, in addition to GalA. IA1-1–2 and IIA1-3–4 also contained GlcA.

Analysis of the neutral fractions IN and IIN. — Each fraction was methylated and the products were fractionated on Sephadex LH-20 to give the fractions of high (INH and IINH) and low molecular weight (INL and IINL). Methylation analysis (Table I) showed that INH and IINH mainly contained terminal and 6-linked Gal, and terminal and 4-linked Glc; branched Rha could not be detected. INL and IINL mainly contained terminal, 4-, 3-, and 6-linked Gal, and terminal Glc in addition to

TABLE I

METHYLATION ANALYSIS OF THE NEUTRAL FRACTIONS FROM GL-PI AND -PII BY BASE-CATALYSED β-ELIMINATION

Glycosyl residue	*Position of OMe groups*	*Position of deuterium*	*Deduced glycosidic linkages*	*Composition (mol %)*			
				INH	*INL*	*IINH*	*IINL*
Ara	2,3,5		terminal (furanosyl)	1.8	4.3	0.9	0.7
	2,3		4 or 5	n.d.[a]	5.5	n.d.	2.2
Rha	2,3,4		terminal	1.7	5.1	1.3	1.2
	3,4		2	n.d.	7.9	n.d.	6.5
	3		2,4	n.d.	1.1	n.d.	0.5
Gal	2,3,4,6		terminal	16.5	19.3	9.2	13.8
	2,3,6		4	5.5	9.5	5.0	19.0
	2,3,4		6	36.8	10.7	40.3	16.1
	2,4,6		3	n.d.	8.9	n.d.	10.2
	2,3		4,6	2.5	5.5	3.1	3.6
	2,4		3,6	n.d.	n.d.	2.8	n.d.
Glc	2,3,4,6		terminal	11.9	11.5	9.2	19.3
	2,3,6		4	9.0	n.d.	11.0	n.d.
	2,3		4,6	2.5	n.d.	3.4	n.d.
GalA	2,3,6	6,6-d_2	4	4.3	7.3	2.3	5.0
GlcA	2,3,4,6	6,6-d_2	terminal	1.3	2.2	n.d.	1.2
	2,3,6	6,6-d_2	4	1.0	n.d.	0.6	n.d.

[a]Not detected.

TABLE II

DIAGNOSTIC IONS IN C.I.- AND E.I.-M.S. OF METHYLATED MONOGLYCOSYLALDITOLS OF INL AND IINL

Fraction	*Fragment*	*Monoglycosylalditol*	*C.i. (relative abundance)*					*E.i. (relative abundance)*							
			$(M+H)^+$	aJ_2	aJ_2OH_2	bA_1	bA_2	aJ_1	aJ_2	bA_1	bA_2	bA_3	*ald*		
INL	**1**	Rha-(1→2)-Rha-ol-*1-d*	412	206	224	189	157	266	206	189	157	125	147	276	308
			(7)	(37)	(46)	(100)	(18)	(1)	(44)	(100)	(34)	(12)	(4)	(0.3)	(0.2)
	2	Rha-(1→4)-Rha-ol-*1-d*	412	206	224	189	157	266	206	189	157	125	134	321	352
			(7)	(37)	(46)	(100)	(18)	(1)	(44)	(100)	(34)	(12)	(4)	(0.3)	(0.2)
	3	Ara-(1→4)-Rha-ol-*1-d*	398	206	224	175	143		206	175	143	111	134	275	350
			(1)	(19)	(17)	(100)	(78)		(14)	(73)	(100)	(7)	(12)	(0.2)	(1.2)
	4	Gal-(1→4)-Rha-ol-*1-d*	442	206	224	219	187	266	206	219	187		134	307	
			(4)	(14)	(19)	(100)	(98)	(8)	(6)	(29)	(100)		(21)	(0.7)	
	5	Gal-(1→6)-Gal-ol-*1-d*	472	236	254	219	187	296	236	219	187	155	134	261	293
			(14)	(8)	(100)	(23)	(98)	(5)	(52)	(11)	(100)	(32)	(4)	(0.9)	(0.5)
IINL	**6**	Rha-(1→2)-Rha-ol-*1-d*	412	206	224	189	157	266	206	189	157	125	147	264	276
			(6)	(35)	(52)	(100)	(15)	(2)	(100)	(100)	(87)	(26)	(10)	(0.5)	(1)
	7	Rha-(1→4)-Rha-ol-*1-d*	412	206	224	189	157	266	206	189	157	125	134	321	277
			(6)	(35)	(52)	(100)	(100)	(2)	(100)	(100)	(87)	(26)	(10)	(0.5)	(1)
	8	Ara-(1→4)-Rha-ol-*1-d*	398	206	224	175	143	266	206	175	143		134	231	307
			(3)	(30)	(29)	(100)	(52)	(2)	(19)	(83)	(100)		(15)	(2)	(0.1)
	9	GlcA-(1→4)-Rha-ol-*1-d*	444	206	224	221	189	266	206	221	189		134	321	353
			(4)	(31)	(38)	(8)	(100)	(6)	(67)	(6)	(100)		(28)	(0.4)	(0.2)
	10	Gal-(1→4)-Rha-ol-*1-d*	442	206	224	219	187	266	206	219	187	155	134	307	382
			(4)	(15)	(24)	(100)	(79)	(16)	(100)	(44)	(100)	(45)	(33)	(0.5)	(0.5)

small proportions of terminal and 4- or 5-linked Ara; terminal, 2-linked, and 2,4-disubstituted Rha; and 4,6-disubstituted Gal. INH, INL, IINH, and IINL gave partially methylated galactitol-*6,6-d*$_2$ and glucitol-*6,6-d*$_2$ acetates. However, the carboxyl-reduction was not performed in the present methylation procedure, and, probably, these *6,6-d*$_2$ derivatives were formed in the β-elimination.

C.i.-m.s. (Table II) showed that INL and IINL each contained five fragments (**1–5** in INL and **6–10** in IINL), which were eluted in the region for glycosylalditols. Fragments **1, 2, 6,** and **7** gave ions at *m/z* 412 [(M + H)$^+$], 206 (aJ$_2$), and 189 (bA$_1$); **3** and **8** at *m/z* 398 [(M + H)$^+$], 206 (aJ$_2$), and 175 (bA$_1$); **4** and **10** at *m/z* 442 [(M + H)$^+$], 206 (aJ$_2$), and 219 (bA$_1$); **9** at *m/z* 444 [(M + H)$^+$], 206 (aJ$_2$), and 221 (bA$_1$); and **5** at *m/z* 472 [(M + H)$^+$], 236 (aJ$_2$), and 219 (bA$_1$), suggesting the presence of the following units: 6-deoxyhexosyl→6-deoxyhexitol-*1-d* in **1, 2, 6,** and **7**, pentosyl→6-deoxyhexitol-*1-d* in **3** and **8**, hexosyl→6-deoxyhexitol-*1-d* in **4** and **10**, hexouronosyl→6-deoxyhexitol-*1-d* in **9**, and hexosyl→hexitol-*1-d* in **5**. From e.i.-m.s. data and the retention times in g.l.c., **1** and **6** were identified as Rha-(1→2)-Rha-ol-*1-d*, **2** and **7** as Rha-(1→4)-Rha-ol-*1-d*, **3** and **8** as Ara-(1→4)-Rha-ol-*1-d*, **4** and **10** as Gal-(1→4)-Rha-ol-*1-d*, and **5** as Gal-(1→6)-Gal-ol-*1-d*. The fragment **9** was eluted faster than the standard glycosylalditol, GalA-(1→4)-Rha-ol-*1-d*, and it was identified as GlcA-(1→4)-Rha-ol-*1-d*. Di- and tri-glycosylalditols were not detected because most of these oligoglycosylalditols are usually lost in splitless injection.

TABLE III

METHYLATION ANALYSIS OF THE ACIDIC FRACTIONS (IA1-2-HMW AND -LMW, AND IIA1-4-HMW AND -LMW) FROM GL-PI AND -PII

Glycosyl residue	*Position of OMe groups*	*Position of deuterium*	*Deduced glycosidic linkages*	*Composition (mol %)*			
				IA1-2		*IIA1-4*	
				H	*L*	*H*	*L*
Ara	2,3,4		terminal (pyranosyl)	n.d.[a]	0.2	n.d.	n.d.
Rha	1,3,4,5	1-*d*	2 (reducing terminal)	n.d.	1.7	n.d.	0.3
	2,3,4		terminal	n.d.	16.6	3.1	10.8
	3,4		2	3.9	12.9	10.9	10.1
	3		2,4	n.d.	n.d.	n.d.	n.d.
Gal	2,3,4,6		terminal	6.2	15.5	9.8	11.9
	2,3,6		4	10.3	9.2	7.1	4.1
	2,3,4		6	48.7	8.7	11.7	n.d.
	2,3		4,6	n.d.	1.4	1.6	0.7
	2,4		3,6	n.d.	n.d.	n.d.	n.d.
Glc	2,3,4,6		terminal	n.d.	1.7	1.8	3.3
GalA	2,3,4,6	6,6-d_2	terminal	n.d.	3.0	2.0	2.0
	2,3,6	6,6-d_2	4	30.9	27.9	51.0	55.3
GlcA	2,3,4,6	6,6-d_2	terminal	n.d.	1.7	0.9	1.7

[a]Not detected.

TABLE IV

METHYLATION ANALYSIS OF ACIDIC FRACTIONS (IA1-1, IIA1-1, IIA1-2, AND IIA1-3) FROM GL-PI AND -PII

Glycosyl residue	*Position of OMe groups*	*Position of deuterium*	*Deduced glycosidic linkages*	*Composition (mol %)*			
				IA1-1	*IIA1-1*	*IIA1-2*	*IIA1-3*
Rha	2,3,4		terminal	5.9	7.6	8.9	8.4
	3,4		2	10.4	8.1	22.0	20.4
	3		2,4	3.6	3.9	2.9	1.7
Gal	2,3,4,6		terminal	9.7	8.2	9.3	10.7
	2,3,6		4	8.6	5.3	6.4	8.1
	2,3,4		6	23.5	12.1	12.5	7.9
	2,3		4,6	n.d.[a]	n.d.	n.d.	n.d.
	2,4		3,6	n.d.	5.9	n.d.	n.d.
Glc	2,3,4,6		terminal	n.d.	n.d.	n.d.	1.4
GalA	2,3,4	6,6-d_2	terminal	n.d.	n.d.	n.d.	2.2
	2,3	6,6-d_2	4	38.3	44.4	38.0	36.5
	2	6,6-d_2	3,4	n.d.	4.4	n.d.	n.d.
GlcA	2,3,4	6,6-d_2	terminal	n.d.	n.d.	n.d.	2.7

[a]Not detected.

Analysis of the acidic fractions from GL-PI and GL-PII. — The acidic fractions IA1-1–2 and IIA1-1–4 were methylated and then carboxyl-reduced with sodium borodeuteride. The products from IA1-2 and IIA1-4 were remethylated and then fractionated on Sephadex LH-20 to give materials of high (IA1-2H and IIA1-4H) and low molecular weight (IA1-2L and IIA1-4L). Methylation analysis (Table III) showed that IA1-2H and IIA1-4H each contained mainly 4-linked GalA and 6-linked Gal. In addition, IA1-2H contained 4-linked Gal, whereas IIA1-4H contained terminal Gal and a larger proportion of 2-linked Rha than IA1-2H. IA1-2L and IIA1-4L mainly contained 4-linked GalA, terminal Gal, and terminal and 2-linked Rha. IA1-2L also contained 4-linked Gal, and IA1-2L and IIA1-4L contained a small proportion of 2-linked rhamnitol-*1-d*. Methylation analysis (Table IV) showed that IA1-1 and IIA1-1–3 mainly contained 4-linked GalA in addition to 2-linked Rha, and 6-linked and terminal Gal. IA1-1 and IIA1-3 also contained a large proportion of 4-linked Gal.

In g.l.c.–m.s. (Table V), methylated IA1-2L and IIA1-4L gave fragments (**11** and **12** from IA1-2L, and **13** and **14** from IIA1-4L) which were eluted in the glycosylalditol region. C.i.-m.s. suggested **11** and **13** to contain a 6-deoxyhexosyl→6-deoxyhexitol-*1-d* unit, and **12** and **14** to contain a hexuronosyl→6-deoxyhexitol-*1-d* unit as described above. In e.i.-m.s., the fragment ions assigned to ald series could not be detected because of their low abundance, so that the glycosidic linkages were not identified. Since IA1-2L and IIA1-4L comprised 2-linked rhamnitol-*1-d*, **11** and **13** are Rha-(1→2)-Rha-ol-*1-d*, and **12** and **14** are HexA-(1→2)-Rha-ol-*1-d*. Fragments **11** and **13** might be formed from 4,5-unsaturated-GalA-(1→2)-Rha-(1→2)-Rha-ol-*1-d* during the methylation because they were the ab-

TABLE V

DIAGNOSTIC IONS IN C.I.- AND E.I.-M.S. OF METHYLATED MONOGLYCOSYLALDITOLS FOR THE PRODUCTS (IA1-2L AND IIA1-4L) FROM GL-PI AND -PII

Fraction	*Fragment*	*Monoglycosylalditol*	*C.i. (relative abundance)*						*E.i. (relative abundance)*			
			$(M+H)^+$	$(M+H)^+ - MeOH$	aJ_2	aJ_2OH_2	bA_1	bA_2	aJ_1	aJ_2	bA_1	bA_2
IAI-2L	**11**	Rha→Rha-ol-*1d*	412 (19.0)		206 (71.0)	224 (32.0)	189 (100)		266 (3.0)	206 (100)	189[a] (100)	
	12	HexA→Rha-ol-*1-d*		412 (17.0)	206 (58.0)	224 (21.0)	221 (12.0)	189 (100)	266 (1.0)	206 (80.0)	221 (9.0)	189 (100)
IIA1-4L	**13**	Rha→Rha-ol-*1-d*	412 (19.0)		206 (60.0)	224 (44.0)	189 (100)		266 (4.0)	206 (100)	189[a] (97.0)	
	14	HexA→Rha-ol-*1-d*		412 (11.0)	206 (44.0)	224 (25.0)	221 (5.0)	189 (100)	266 (3.0)	206 (100)	221 (8.0)	189 (100)

[a]This peak, when analysed by high-resolution mass spectrometry, had *m/z* 189.1122 assigned to $C_9H_{17}O_4$.

TABLE VI

DIAGNOSTIC IONS IN E.I.-M.S. OF METHYLATED DIGLYCOSYLALDITOLS FOR THE PRODUCTS (IA1-2L AND IIA1-4L) FROM GL-PI AND -PII

Fraction	*Fragment*	*Diglycosylalditol*	*E.i.-m.s.* [m/z *(relative abundance)*]						
			cA_1	cA_2	aJ_1	aJ_2	cbA_1	cbA_2	abJ_2
IA1-2L	**15**	HexA→Rha→Rha-ol-*1-d*	221 (21.0)	189 (100)	266 (2.0)	206 (88.0)	395 (3.0)	363 (10.0)	
IIA1-4L	**16**	HexA→Rha→Rha-ol-*1-d*	221 (18.0)	189 (100)		206 (12.0)	395 (4.0)	363 (8.0)	380 (2.0)
	17	HexA→Rha→Rha-ol-*1-d*	221 (20.0)	189 (100)	266 (1.0)	206 (78.0)	395 (2.0)	363 (8.0)	380 (1.0)

sorbed fractions on DEAE-Sephadex, and IA1-2L and IIA1-4L mainly contained 2-linked Rha. In g.l.c.–m.s. (Table VI), IA1-2L and IIA1-4L also gave fragments (**15** from IA1-2L, and **16** and **17** from IIA1-4L). In c.i.-m.s., these fragments were not detected because of the low sensitivity. The e.i.-m.s. data suggested that these fragments were HexA→Rha→Rha-ol-*1-d*. In g.l.c., **15** and **17** had the same retention times, whereas **16** was eluted faster than **15** and **17**. Although the glycosidic linkages in **15**–**17** could not be deduced, it was assumed that either the glycosidic linkages or the HexA component in **15** and **17** differed from those in **16**.

Digestion of GL-PIV with endo-α-(1→4)-polygalacturonase. — Previous results suggested[1] that GL-PIV contained a large polygalacturonan moiety and a small rhamnogalacturonan moiety, hence, it was digested with endo-α-(1→4)-polygalacturonase. Elution of the products from Sephadex G-50 gave a small proportion of a fraction (PG-1) in the void volume, an intermediate fraction (PG-2), and a fraction (PG-3) of the lowest molecular weight (Fig. 4). PG-1–2 contained Rha, Ara, Gal, and Glc in the molar ratios 0.1:trace:1.0:0.07 and 0.3:trace:1.0:0.3, respectively. PG-2 also contained a large proportion of GalA and a small proportion of GlcA. PG-3 comprised mainly GalA, in addition to a small proportion of Rha, Ara, and Gal in the molar ratios trace:0.2:1.0. These results suggested that PG-1 was attached to PG-2 and PG-3 through a (1→4)-α-D-galacturonan moiety.

Analysis of the products of enzymic digestion of GL-PIV. — PG-1–2 were each methylated, then carboxyl-reduced with sodium borodeuteride, and the products were converted into the alditol acetates. Methylation analysis (Table VII) showed that PG-1 mainly contained terminal, 6-linked, and 4,6-disubstituted Gal, and 4-linked Glc. PG-2 contained large proportions of terminal and 4-linked GalA in addition to terminal Gal. PG-1 also contained small proportions of 2-linked and 2,4-disubstituted Rha, and 2,4-branched GalA, whereas PG-2 comprised 2- and

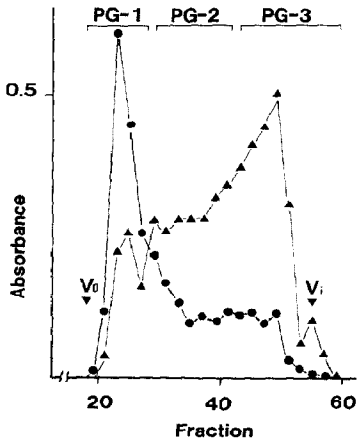


Fig. 4. Gel filtration on Sephadex G-50 of the products from GL-PIV after digestion with endo-α-(1→4)-polygalacturonase: ●, carbohydrate (490 nm); ▲, uronic acid (520 nm).

TABLE VII

METHYLATION ANALYSIS OF PRODUCTS FROM GL-PIV BY ENDO-α-(1→4)-POLYGALACTURONASE DIGESTION

Glycosyl residue	*Position of OMe groups*	*Position of deuterium*	*Deduced glycosidic linkages*	*Composition (mol %)*			
				PG-1[a]	*PG-2*[a]	*PG-3H*[b]	*PG-3L*[b]
Ara	2,3,5		terminal (furanosyl)	n.d.[c]	0.4	n.d.	n.d.
	2,3,4		terminal (pyranosyl)	2.2	0.8	n.d.	n.d.
Rha	2,3,4		terminal	n.d.	n.d.	n.d.	n.d.
	3,4		2	5.7	4.3	n.d.	n.d.
	2,4		3	n.d.	1.8	n.d.	n.d.
	3		2,4	0.9	4.1	n.d.	n.d.
Fuc	2		3,4	n.d.	4.5	n.d.	n.d.
Gal	2,3,4,6		terminal	9.7	8.5	7.2	12.1
	2,3,6		4	3.5	n.d.	1.4	n.d.
	2,4,6		3	7.0	n.d.	n.d.	n.d.
	2,3,4		6	9.9	6.6	11.1	n.d.
	3,6		2,4	n.d.	3.8	n.d.	n.d.
	2,3		4,6	12.6	3.2	n.d.	3.1
	2,4		3,6	6.7	2.1	n.d.	n.d.
	2		3,4,6	5.0	1.3	n.d.	n.d.
	3		2,4,6	1.4	0.6	n.d.	n.d.
GalA	2,3,4	6,6-d_2	terminal	n.d.	13.3	n.d.	n.d.
	2,3	6,6-d_2	4	1.9	28.6	n.d.	n.d.
	2	6,6-d_2	3,4	n.d.	5.4	n.d.	n.d.
	3	6,6-d_2	2,4	0.2	3.8	n.d.	n.d.
	1,2,3,5,6	1,6,6-d_3	4 (reducing terminal)			n.d.	11.0
	2,3,4,6	6,6-d_2	terminal			43.3	30.3
	2,3,6	6,6-d_2	4			34.6	43.5
GlcA	2,3,4	6,6-d_2	terminal	7.0	2.0	n.d.	n.d.
	2,3	6,6-d_2	4	4.7	n.d.	n.d.	n.d.
Glc	2,3,4,6		terminal	7.7	n.d.	n.d.	n.d.
	2,3,6		4	8.8	3.8	n.d.	n.d.
	2,6		3,4	2.9	1.2	n.d.	n.d.
	4,6		2,3	2.3	n.d.	n.d.	n.d.

[a]Samples were methylated, carboxyl-reduced with sodium borodeuteride in tetrahydrofuran–ethanol, and then acetylated. [b]Samples were reduced with carbodi-imide and sodium borodeuteride in deuterium oxide, methylated, and then acetylated. [c]Not detected.

3-linked Rha, 2,4-disubstituted Rha, 3,4-disubstituted Fuc, and 3,4- and 2,4-disubstituted GalA.

PG-3 was carboxyl-reduced with sodium borodeuteride in the presence of a water-soluble carbodi-imide, and the product was fractionated on Bio-gel P-2 (data not shown) into equal proportions of material of high (PG-3H) and low molecular weight (PG-3L). PG-3H and PG-3L were each reduced with sodium borodeuteride and then methylated. Methylation analysis (Table VII) revealed large proportions of terminal and 4-linked GalA. PG-3H also contained 6-linked Gal, whereas PG-3L comprised 4-linked galactitol-1,6,6-d_3 and terminal Gal. G.l.c.–m.s. (Table VIII and IX) suggested that PG-3L contained GalA-(1→4)-GalA (**19**), GalA→Rha→GalA (**21**), and GalA-(1→4)-[GalA-(1→2)-GalA] (**22**). PG-3L also contained Gal-

TABLE VIII

DIAGNOSTIC IONS IN C.I.- AND E.I.-M.S. OF METHYLATED MONOGLYCOSYLALDITOLS FROM PG-3L

Fragment	*Monoglycosylalditol*	*C.i. (relative abundance)*					*E.i. (relative abundance)*							
		$(M+H)^+$	aJ_2	aJ_2OH_2	bA_1	bA_2	aJ_1	aJ_2	bA_1	bA_2	bA_3	*ald*		
18	Gal-(1→4)-Rha-ol-*1-d*	442	206	224	219	187	266	206	219	187	155	134	275	382
		(1.0)	(11.0)	(65.0)	(21.0)	(47.0)	(5.0)	(47.0)	(17.0)	(100)	(6.0)	(22.0)	(0.7)	(1.0)
19	GalA-(1→4)-GalA-ol-*1-d*	476	238	256	221	189	298	238	221	189	157	134	341	384
		(19.0)	(16.0)	(69.0)	(66.0)	(100)	(17.0)	(100)	(54.0)	(100)	(61.0)	(33.0)	(2.0)	(2.0)

TABLE IX

DIAGNOSTIC E.I.-M.S. IONS OF METHYLATED DIGLYCOSYLALDITOLS FROM PG-3L

Fragment	*Diglycosylalditol*	*E.i.-m.s. fragment ions* [m/z *(relative abundance)*]									
		aJ_1	aJ_2	cA_1	cA_2	abJ_1	abJ_2	cbA_1	cbA_2		
20	Rha→Rha→GalA-ol-*1-d*	298	238	189	157		412		331		
		(8.0)	(73.0)	(16.0)	(31.0)		(3.0)		(2.0)		
21	GalA→Rha→GalA-ol-*1-d*	298	238	221	189		412	395	363		
		(5.0)	(44.0)	(33.0)	(100)		(3.0)	(2.0)	(2.0)		
		bA_1	bA_2	$b'A_1$	$b'A_2$	abJ_1	abJ_2	$ab'J_1$	$ab'J_2$	*ald*	
22	GalA-(1 ↘ 4) GalA-ol-*1-d* ↗ 2) GalA-(1	221	189	221	189	504	444	504	444	341	296
		(100)	(9.0)	(100)	(9.0)	(8.0)	(1.0)	(8.0)	(1.0)	(17.0)	(10.0)

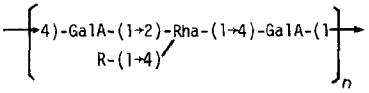
(1→4)-Rha (**18**) and Rha→Rha→GalA (**20**). The endo-α-polygalacturonase from *A. niger* hydrolyses polygalacturonic acid into mono- to tri-galacturonides[25]. Since the enzyme cannot hydrolyse such linkages as GalA→Gal and GalA→Rha, the enzyme preparation might be contaminated with a trace of exo-galacturonidase.

DISCUSSION

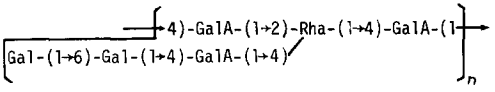
The potent anti-complementary polysaccharides, GL-PI, GL-PII, and GL-PIV, isolated from the leaves of *P. ginseng*, have been characterised[1] as acidic pectic polysaccharides. GL-PI and GL-PII contained Rha (2-linked and 2,4-di-substituted) and 4-linked GalA in the molar ratios of 30.9:38.6 and 30.9:30.8, respectively, and the backbones of GL-PI and GL-PII were shown to be mainly rhamnogalacturonan regions with little galacturonan. GL-PIV contained much more 4-linked GalA than 2-linked and 2,4-disubstituted Rha, and the backbone was shown to be rich in the galacturonan. The backbones were shown to be substituted[1] by neutral side-chains mostly attached at position 4 of 2-linked Rha or positions 2 or 3 of 4-linked GalA.

On β-eliminative degradation, GL-PI and -PII gave neutral (IN and IIN) and acidic (IA and IIA) fractions of which the former contained neutral side-chains (INH, IINH, **2–5, 7, 8,** and **10**) and the fragments (**1** and **6**) derived from the backbones. Structural analysis suggested that the rhamnogalacturonan cores of GL-PI and -PII were comprised of dirhamnosyl units because Rha-(1→2)-Rha-ol-*1-d* (**1, 6, 11,** and **13**) were detected in the products.

GL-PI and -PII possessed Rha, Ara, and Gal as the neutral side-chains attached to position 4 of 2-linked Rha in the rhamnogalacturonan cores (**23**). GL-PI and -PII had Gal-(1→6)-Gal as the side chain originally attached to position 4 of GalA as Gal-(1→6)-Gal-(1→4)-GalA-(1→ because Gal-(1→6)-Gal-ol-*1-d* was released by β-eliminative degradation. Some pectic polysaccharides contain[4,5,26,27] the sequence →4)-GalA-(1→4)-Rha-(1→, and it has been proposed[7,15,26,27] that some galactosyl chains are attached to position 4 of GalA which, in turn, is attached to position 4 of 2-linked Rha in the rhamnogalacturonan core. Therefore, Gal-(1→6)-Gal in GL-PI and GL-PII is attached to the rhamnogalacturonan cores in the same



23 R=Rha, Ara, or Gal



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manner (**24**). GL-PI and -PII also contained long (1→6)-linked galactosyl chains in addition to small proportions of (1→4)-linked glucosyl chains. The reducing terminals of these side chains could not be detected, and it is not known whether they were attached to position 4 of 2-linked Rha in the rhamnogalacturonan cores either directly (**23**) or through 4-linked GalA (**24**).

The acidic fractions (IA and IIA) from GL-PI and -PII mainly contained the rhamnogalacturonan core and (1→6)-linked galactosyl chains. The structural analysis of the neutral side-chains in anti-complementary pectic polysaccharides, AR-2IIa–IId, from *Angelica acutiloba* indicated[27] that the acidic fractions derived by β-eliminative degradation are formed by incomplete β-elimination. Furthermore, the structural analysis of the acidic fractions also suggests[27] strongly the presence of galactosyl chains which were attached originally to GalA as in →4)-GalA-(1→$(Gal)_n$-(1→4)-GalA-(1→. Thus, it is assumed that IA and IIA might be formed by incomplete β-elimination. The reducing terminals in IA and IIA could not be detected, but it was assumed that (1→6)-linked galactosyl chains in IA and IIA might have been attached originally to GalA as in →4)-GalA-(1→$(Gal)_n$-(1→4)-GalA-(1→ or →4)-GalA-(1→$(Gal)_n$-(1→4)-Rha-(1→.

Endo-α-(1→4)-polygalacturonase degraded GL-PIV into PG-1–3. Methylation analysis showed that PG-3 was mainly comprised of oligogalacturonides, indicating that PG-1 and PG-2 were attached to each other through galacturonans that were degraded to oligogalacturonides by the enzyme. PG-1 mainly contained rhamnogalacturonan core as the backbone, to which were attached galactosyl chains that comprised terminal, 6-linked, and 4,6-disubstituted Gal, and (1→4)-linked glucosyl chains. PG-2 contained a large proportion of (1→4)-α-galacturonan partially branched at position 2 or 3, in addition to a small proportion of the rhamnogalacturonan core and various galactosyl chains. Of the galactosyl chains, about two-thirds were attached to position 2 or 3 of galacturonan and the remainder to the rhamnogalacturonan core.

It has been proposed[28] that the anti-complementary activities of the "ramified" regions from pectic polysaccharides (AR-2IIa–IId) of *A. acutiloba* are expressed by a combination of the rhamnogalacturonan core and (1→6)-linked galactosyl chains, and suggested[7] that (1→6)-linked galactosyl chains might be essential for the expression of the activity. GL-PI and GL-PII consisted mainly of (1→6)-linked galactosyl chains as the side chain and, therefore, may express anti-complementary activity in a manner similar to that of AR-2IIa–IId. However, GL-PIV contains (1→4,6)-linked galactosyl chains in addition to short galactosyl chains consisting of 3-, 4-, and 6-linked Gal. Pectin[29] from the fruit of *Zyziphus jujuba*, which has been reported[30] to have no anti-complementary activity, mainly contains[29] 4-linked Gal as neutral sugar. An anti-complementary inactive arabinogalactan (AGIIb-2) has also been isolated[31] from *A. acutiloba*, which consists of a (1→4)-galactan possessing (1→6)-linked galactosyl side-chains at position 6 (unpublished data). The relationship between structure and activity of GL-PIV must await further study.

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