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

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ABSTRACT

Four anti-complementary polysaccharides, GL-PI–GL-PIV, isolated from the leaves of *Panax ginseng* C. A. Meyer showed anti-complementary activities in the sequence I>II≈IV≫III. GL-PI had the highest molecular weight, 50,000 by h.p.l.c. (0.2M sodium chloride). GL-PI and GL-PII consisted mainly of Rha, Gal, and GalA, and GL-PIII contained Fuc in addition, whereas GL-PIV consisted mainly of Gal, Glc, and GalA. The ^{1}H- and ^{13}C-n.m.r. spectra of GL-PII indicated the Gal, Rha, and GalA to be β, α, and α, respectively. GL-PI and GL-PII contained mainly (1→2)-linked Rha*p* and (1→4)-linked GalA. GL-PII contained, in addition, 2,4-di-*O*-substituted Rha*p*, non-reducing terminal Gal*p*, and (1→6)-linked Gal*p* as the major glycosidic linkages. GL-PIV also contained mainly (1→4)-linked GalA, non-reducing terminal Gal*p*, and (1→6)-linked Gal*p*, whereas GL-PIII contained mainly non-reducing terminal GalA, and 3,4-di-*O*- and 2,4-di-*O*-substituted GalA, in addition to (1→4)-linked GalA and (1→2)-linked Rha*p*. The results of base-catalysed β-elimination indicated that these four polysaccharides have a rhamnogalacturonan backbone consisting of 4-linked GalA and 2-linked Rha*p*. Some 6-linked Gal*p* was attached to GalA or GlcA.

INTRODUCTION

The root of *Panax ginseng* C. A. Meyer is a well known Chinese drug used clinically for stomach disorders and as a tonic, *etc.* Recently, several pharmacologically active polysaccharides have been isolated from the roots of *P. ginseng* C. A. Meyer[1–3] and the related species, *P. notoginseng* (Burk.) F. H. Chen[4]. Although the polysaccharide fraction from the roots of *P. ginseng* has been used clinically in China for the treatment of cancer, little has been reported on polysaccharides isolated from the leaves. Recently, Yamada *et al.*[5–14] reported that

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several plant polysaccharides have anti-complementary activity. Some anti-tumor polysaccharides also have anti-complementary activity[15].

We now report on anti-complementary polysaccharides isolated from the leaves of *P. ginseng*.

EXPERIMENTAL

Materials. — The leaves of *Panax ginseng* C. A. Meyer, which were cultivated in Jilin, were collected on Chang Bai mountain, China. DEAE-Sephadex A-50, Sephadex LH-20, and Sepharose CL-6B were purchased from Pharmacia, and DEAE-TOYOPEARL was from Toyo Soda Co. Ltd. (Japan). Standard pullulans were purchased from Showa Denko Co. Ltd. (Japan). AR-arabino-galactans were prepared by the method of Yamada *et al.* [5,6].

General methods. — Total carbohydrate, uronic acid, protein, and pentose were assayed by the phenol–sulfuric acid[16], *m*-hydroxybiphenyl[17], Lowry[18], and phloroglucinol–acetic acid methods[19], respectively, using Rha or a 1:1 mixture of Rha and Ara, GalA, bovine serum albumin, and Ara as the respective standards. T.l.c. was performed on cellulose (Merck, 5577) in ethyl acetate–pyridine–acetic acid–water (5:5:1:3). Reducing sugars and uronic acid were detected with alkaline silver nitrate[20] and *p*-anisidine hydrochloride[21]. 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. Uronic acids were converted into the corresponding reduced products[22] and analysed by g.l.c. as the alditol acetates. The molar ratios of neutral sugars were calculated from the peak areas and molecular weights of the corresponding alditol acetates. The molar ratios of uronic acid and neutral sugars were calculated from the content of uronic acid. H.p.l.c. was performed[10] on a Waters model 441 equipped with a u.v. detector and differential refractometer. The molecular weight of each polysaccharide was analysed by h.p.l.c. equilibrated with water or 0.2M sodium chloride on an Asahi-pak GS-510 + GS-320 column (Asahi Chemical Industry Co. Ltd., Japan), and was estimated from the calibration curve obtained by using standard pullulans.

Isolation of water-soluble polysaccharides. — The leaves of *Panax ginseng* C. A. Meyer (5 kg) were extracted with water for 3 h at 100°, and the residual material was re-treated twice in this manner. The extracts were combined, concentrated to 3 L by rotary evaporation, and diluted with ethanol (4 vol.) to give GL-1 (140 g). An aqueous solution of GL-1 was dialysed against running water for 8 days, then centrifuged, and diluted with ethanol (4 vol.). An aqueous solution of the precipitate was lyophilised to give GL-2 (14 g). GL-2 was treated thrice with boiling methanol (1 L) for 1 h and the methanol-insoluble portion was lyophilised to give delipidised GL-2 (11.9 g). To an aqueous solution of delipidised GL-2 was added aqueous 8% cetyltrimethylammonium bromide (Cetavlon)[5]. After storage for 6 h at 20°, the precipitate was collected by centrifugation, and a solution in aqueous

10% sodium chloride was diluted with ethanol (3 vol.). The precipitate was dialysed against water and then lyophilised to give an acidic fraction GL-3 (2.5 g). Aqueous 1% boric acid was added to the supernatant solution and the pH was adjusted to 8.8 with 2M sodium hydroxide. After stirring for 2 h, the solution was centrifuged and the precipitate was treated as for GL-3 to give GL-4 (4.4 g). The remaining supernatant solution was acidified with acetic acid and diluted with ethanol (3 vol.) together with potassium acetate. The resulting precipitate was dialysed and then lyophilised to give polysaccharide fraction GL-5 (3 g).

Fractionation of GL-3 to give GL-PI–GL-PIV. — *(a) Ion-exchange chromatography.* GL-3 (2.4 g) was applied to a column (5.5 × 47 cm) of DEAE-Sephadex A-50 (HCO_3^- form) equilibrated with water. The crude acidic polysaccharides, IIa (13.5 mg), IIb (780 mg), IIc (237 mg), IId (89 mg), IIe (134 mg), and IIf (112 mg) were eluted with 0.1, 0.3, 0.4, 0.5, 0.7, and 1M ammonium hydrogencarbonate, respectively. Each fraction was eluted until sugar was no longer detected, then dialysed and lyophilised. IIb was divided into IIbA (670 mg) and IIbB (110 mg) according to the elution pattern. IIbA (280 mg) was washed through a Sep-Pak C_{18} cartridge with water to remove the coloured material, then applied to a column (5.5 × 40 cm) of DEAE-TOYOPEARL 650C (Cl^- form) equilibrated with 2mM sodium chloride. IIbA-1 (39 mg) was eluted with 0.1M sodium chloride, and IIbA-2 (91 mg) and IIbA-3 (69 mg) with a linear gradient elution of 0.1→0.3M sodium chloride (500 mL).

(b) Ethanol fractionation. IIbA-2 and IIbA-3 were fractionated by dilution of aqueous solutions with ethanol to 62.5 and 66.7%, respectively. The precipitates and supernatant solutions were purified by gel filtration on Sepharose CL-6B, respectively. A purified acidic polysaccharide, GL-PIV (yield, 12 mg) was obtained from IIbA-1 after the ethanol precipitation.

(c) Gel-filtration chromatography. This was performed on a column (2.8 × 94 cm) of Sepharose CL-6B, equilibrated with 0.2M sodium chloride. The purity of each fraction was monitored by h.p.l.c. The eluates which showed a single peak and the same retention time were combined (Fig. 2), and the purified acidic polysaccharides, GL-PI (25 mg), GL-PII (40 mg), and GL-PIII (11.2 mg) were obtained.

Methylation analysis. — Each polysaccharide was methylated once by the Hakomori method[23] in order to prevent β-elimination, and the completeness of the methylation was checked by using triphenylmethane[24]. Each methylated polysaccharide was purified by using a Sep-Pak C_{18} cartridge (Waters Assoc.)[25], and then a solution in 95% ethanol–tetrahydrofuran[26] (27:73) was reduced with sodium borodeuteride. The resulting carboxyl-reduced, partially *O*-methylated polysaccharide was hydrolysed (2M trifluoroacetic acid, 1 h, 121°), and the products were reduced and acetylated. The resulting alditol acetates were analysed by g.l.c. and g.l.c.–m.s.[27] G.l.c. was performed on a Hewlett-Packard model 5840A gas chromatograph equipped with a flame-ionisation detector. A solution of partially methylated alditol acetates in acetone was injected[11] into a DB-1 capillary column

(30 m × 0.25 mm i.d., 0.25-μm film; J and W Scientific Inc.) with split-less injection. G.l.c.–m.s. (70 eV) was performed[12] on a JEOL DX-300 instrument equipped with a SPB-1 capillary column (25 m × 0.25 mm i.d., 0.25-μm film; SPELCO). Peaks were identified on the basis of relative retention time and fragmentation patterns. The molar ratios for each sugar were calibrated using the peak areas and response factors[28] of the flame-ionisation detector in g.l.c. on DB-1.

N.m.r. studies. — The ^{1}H- (400 MHz) and ^{13}C-n.m.r. (100 MHz) spectra of GL-PII were obtained for a 0.5% solution in D_2O at 80°, using a Varian XL-400 F.t. spectrometer. Chemical shifts were expressed relative to that of sodium 3-(trimethylsilyl)propane-1-sulphonate-d_4 (TSP).

β-Elimination of the methylated acidic polysaccharides[29,30]. — To solutions of dry methylated GL-PI–GL-PIV (2 mg each) in methyl sulfoxide (1 mL) was added methylsulfinylmethanide (250 μL), and each mixture was stirred for 24 h at room temperature. To half of each sample was added excess of ethyl iodide, the mixture was kept overnight at room temperature and then concentrated, and the product was recovered by using a Sep-Pak C_{18} cartridge and the procedure of Waeghe *et al.*[25], except that the samples were eluted with ethanol. The ethylated samples were fractionated[12] on a column (1.0 × 25 cm) of Sephadex LH-20 equilibrated with chloroform–methanol (1:1), and fractions of high (I–IV/R_2-a) and low molecular weight (I–IV/R_2-b) were obtained (detection with the 1-naphthol–sulfuric acid reagent[31]). The various fractions were hydrolysed with 2M trifluoroacetic acid for 1.5 h at 121°, and the products were reduced with sodium borohydride and then acetylated. The remaining half of each sample was neutralised with aqueous 50% acetic acid, and the product (R_1) was recovered as described above, hydrolysed with 2M trifluoroacetic acid (121° for 1.5 h), then reduced, and acetylated to obtain partially *O*-methylated alditol acetates. The partially alkylated alditol acetates were analysed by g.l.c. and g.l.c.–m.s. (as above).

Anti-complementary activity. — This was measured as described previously[5] except that water was used for dilutions.

RESULTS

Purification of polysaccharides. — A crude polysaccharide fraction (GL-2) was obtained from the leaves of *P. ginseng* C. A. Meyer by extraction with hot water followed by precipitation with ethanol, and dialysis. After extraction of GL-2 with methanol, it was fractionated into GL-3, GL-4, and GL-5 using Cetavlon. The highly acidic polysaccharide fraction (GL-3), which had the highest anti-complementary activity (data not shown), was further fractionated on DEAE-Sephadex (HCO_3^-) into the absorbed fractions IIa–f which were eluted stepwise with 0.1, 0.3, 0.4, 0.5, 0.7, and 1M ammonium hydrogencarbonate, respectively; there was no unabsorbed fraction. IIbA (a sub-fraction of IIb) had the highest anti-complementary activity (data not shown) and the yield was sufficient for a study of the structure. IIbA was eluted as a single peak (monitoring of hexose) from Sepharose

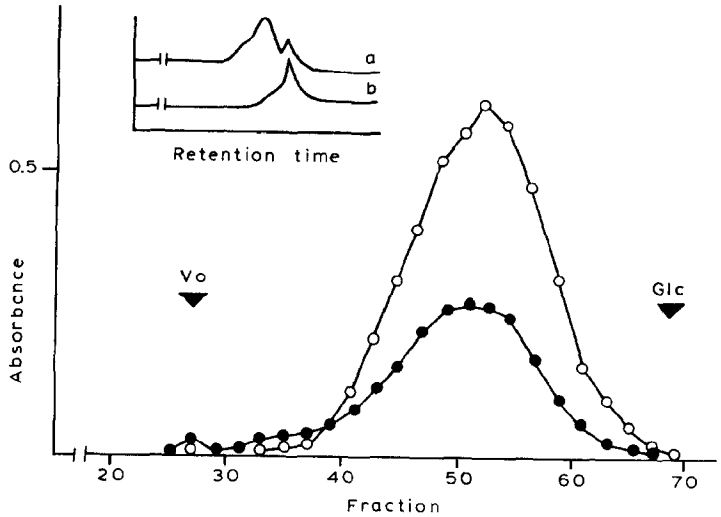

Fig. 1. Gel filtration of IIbA on Sepharose CL-6B: Vo, void volume; Glc, glucose; —○—, uronic acid (520 nm); —●—, carbohydrate (490 nm). Inset, h.p.l.c. of IIbA: a, refractometer response (R.I.); b, 254 nm.

CL-6B which was equilibrated in 20mM acetate buffer (pH 5.6), and the uronic acid was also co-eluted with hexose peak (Fig. 1). IIbA gave a single spot on glass-fiber paper electrophoresis (pyridine–acetate buffer, pH 5.4). Because IIbA had a pale-brown colour, it was passed through a Sep-Pak C_{18} cartridge. However, h.p.l.c. of the decoloured IIbA on Asahi-pak GS-510 + GS-320 revealed heterogeneity (Fig. 1, inset). Therefore, IIbA was further fractionated on DEAE-TOYOPEARL (Cl^-) into IIbA-1, IIbA-2, and IIbA-3 as the bound fractions. IIbA-1 was eluted as a single peak, and recovered as GL-PIV, but IIbA-2 and IIbA-3 were still heterogeneous by h.p.l.c. When the major fraction in IIbA-2, obtained by precipitation with ethanol, was purified on Sepharose CL-6B, a single broad carbohydrate peak was co-eluted with uronic acid (Fig. 2a). Each fraction was subjected to h.p.l.c., and only higher-molecular-weight fractions were detected as a single peak (GL-PII) (Fig. 2a, inset). When IIbA-3 was fractionated by precipitation with ethanol, chromatography of the supernatant solution and the precipitate on Sepharose CL-6B resulted in a broad elution pattern (Figs. 2b and 2c). Each fraction was subjected to h.p.l.c., and GL-PIII and GL-PI were isolated as homogeneous polysaccharides (Figs. 2b and 2c, inset). GL-PI had the highest anti-complementary activity, GL-PII and GL-PIV had almost the same activities, and GL-PIII had the weakest activity (Table I).

Chemical properties of polysaccharides. — The properties of GL-PI–GL-PIV are summarised in Table II. They contained 32–44% of uronic acid and 0.5–1% of protein. A trace of methyl ester was detected in GL-PIII and GL-PIV. GL-PI and GL-PII consisted mainly of Rha, Gal, and GalA in the molar proportions 2.0:1.0:1.8 and 1.1:1.0:1.2, respectively. GL-PIII consisted mainly of Rha, Fuc,

TABLE I

THE ANTI-COMPLEMENTARY ACTIVITIES OF GL-PI–GL-PIV

Sample	*Concentration (μg/mL)*		
	1000	*500*	*100*
	Anti-complementary activity (%)		
I	84.6	81.5	51.3
II	72.5	66.5	33.3
III	24.2	17.8	4.7
IV	71.5	67.6	35.3
AR-arabinogalactans[a]	82.3	61.9	27.8

[a]Positive control.

Gal, and GalA (1.1:1.0:1.4:1.6), whereas GL-PIV consisted mainly of Rha, Gal, Glc, GalA, and GlcA (1.0:3.9:1.5:5.1:1.0). Using h.p.l.c. with 0.2M sodium chloride, GL-PI was shown to have the highest molecular weight (~50,000). However, on elution with water, their molecular weights increased to 250,000–380,000, reflecting self-aggregation.

N.m.r. studies. — The ^{1}H-n.m.r. spectrum (Fig. 3a) of the major anti-complementary polysaccharide, GL-PII, contained signals at 1.2 (Me of Rha), 2.2 (OAc), and 4.6, 5.0, and 5.3 p.p.m. (anomeric protons corresponding[32] to β-D-Gal*p*, α-L-Rha*p*, and α-D-Gal*p*A, respectively). The ^{13}C-n.m.r. spectrum (Fig. 3b) contained signals due to anomeric carbons at 100.39, 100.41, and 106.31 p.p.m., which were assigned[33–35] to α-D-Gal*p*A, α-L-Rha*p*, and β-D-Gal*p* (*cf.* 106.58 p.p.m.

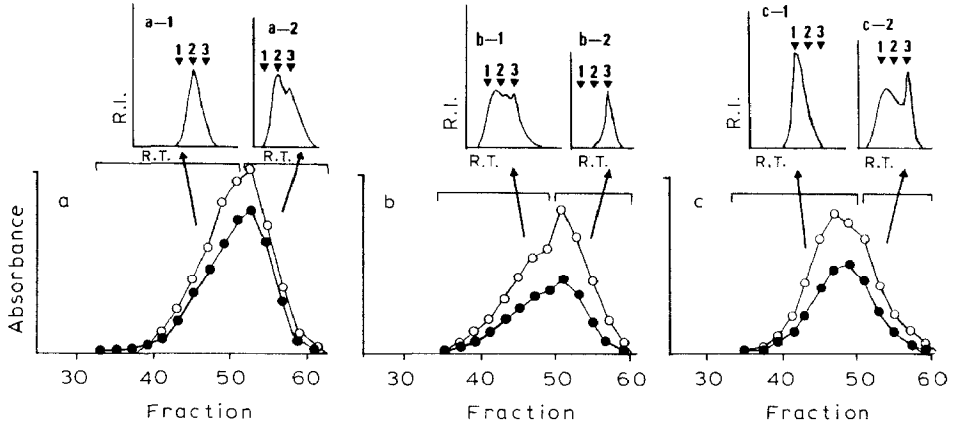

Fig. 2. Gel filtration of (*a*) IIbA-2 ethanol precipitate, (*b*) IIbA-3 ethanol precipitate, and (*c*) IIbA-3 ethanol supernatant solution on Sepharose CL-6B. Inset, h.p.l.c. of the sub-fractions: a-1, fractions 32–51 (GL-PII); a-2, fractions 52–61; b-1, fractions 34–49; b-2, fractions 50–61 (GL-PIII); c-1, fractions 31–50 (GL-PI); c-2, fractions 51–61; R.T., retention time; R.I., refractive index. 1–3 indicate the positions of elution of GL-PI, GL-PII, and GL-PIII, respectively.

TABLE II

PHYSICOCHEMICAL PROPERTIES OF GL-PI–GL-PIV

Chemical properties	*I*	*II*	*III*	*IV*
Carbohydrate (as Rha) (%)	55.0	53.0	40.0	48.8
(as Rha:Gal = 1:1)	n.d.[a]	n.d.[a]	30.0	37.5
Uronic acid (as GalA)	38.7	38.2	31.8	43.8
Protein (as BSA)	0.5	1.0	1.0	1.0
Pentose (as Ara)	n.d.[a]	n.d.[a]	4.2	5.7
Methyl ester (as MeOH)	nil	nil	0.3	0.8
Component sugars (mol %)				
Rha	38.8	30.9	19.0	7.2
Ara	trace	trace	4.0	4.2
Fuc			17.4	4.0
Xyl			trace	1.6
Gal	18.8	27.0	25.0	27.8
Glc	5.6	2.5	3.3	11.1
GalA	34.3	32.7	28.0	36.8
GlcA	2.4	5.8	2.5	7.0
Mol. wt.				
Eluent 0.2M NaCl	50,000	24,000	10,000	6,000
water	300,000	250,000	380,000	380,000

[a]Not determined.

for C-1 of methyl β-D-galactopyranoside)[11], respectively. The signals at 177.55 and 184.81 p.p.m. were assigned to C-6 of Gal*p*A. The methyl signals (C-6) of Rha were observed at 19.48 and 22.88 p.p.m., and the latter may be due to 2,4-di-*O*-substituted Rha.

Methylation analysis. — GL-PI–GL-PIV were each methylated by the method of Hakomori[23] and then hydrolysed with acid, and the products were converted into the partially methylated alditol acetates which were identified by g.l.c.–m.s. (Table III). GL-PI contained mainly 2-*O*-linked Rha*p* and 4-*O*-linked GalA in addition to small proportions of 2,4-di-*O*-substituted Rha*p*, non-reducing-end Gal*p*, 6-*O*-linked Gal*p*, and 4-*O*-linked Gal*p*. GL-PII contained larger proportions of 2,4-di-*O*-substituted Rha*p*, non-reducing-end Gal*p*, 6-*O*-linked Gal*p*, and 4-*O*-linked Gal*p* than GL-PI, in addition to 2-*O*-linked Rha*p* and 4-*O*-linked GalA as the main sugars. In contrast, GL-PIII contained mainly 2-*O*-linked Rha*p*, non-reducing-end GalA, 4-*O*-linked GalA, and 3,4- and 2,4-di-*O*-substituted GalA in addition to small proportions of non-reducing-end Gal*p*, 2,4-di-*O*-substituted Gal*p*, terminal GlcA, 3,4-di-*O*-substituted Fuc*p*, terminal Rha*p*, 2,4-di-*O*-substituted Rha*p*, 4-*O*-linked Gal*p*, and 6-*O*-linked Gal*p*. GL-PIV also contained mainly 4-*O*-linked GalA, large proportions of non-reducing-end Gal*p* and 6-*O*-linked Gal*p*, and small proportions of non-reducing-end Ara*f* and Ara*p*, 3,4-di-*O*-substituted

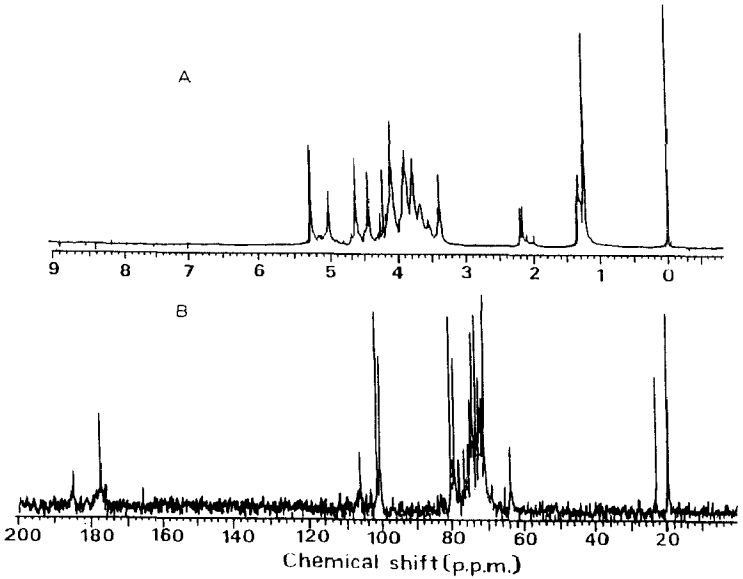

Fig. 3. N.m.r. spectra of GL-PII: A, ^{1}H; B, ^{13}C; recorded relative to internal TSP.

Fuc*p*, 2-*O*-linked Rha*p*, 2,4-di-*O*-substituted Rha*p*, 3-*O*-linked Gal*p*, 3,6-di-*O*-substituted Gal*p*, 3,4,6-tri-*O*-substituted Gal*p*, terminal GalA, 3,4-di-*O*-substituted GalA, 2,4-di-*O*-substituted GalA, 4-*O*-linked Glc*p*, 3,4-di-*O*-substituted Glc, 4-*O*-linked GlcA, and 3,4-di-*O*-substituted GlcA.

Identification of rhamnogalacturonan core by base-catalysed β-elimination. — GL-PI–GL-PIV were each methylated and then subjected to base-catalysed β-elimination using methylsulfinylmethanide. The hydroxyl groups exposed in each elimination product were ethylated to give the products of high (R_2-a) and low molecular weight (R_2-b) isolated using Sephadex LH-20. Methylation analysis of neutral residues in each β-elimination product (R_1) (Table IV) showed a decrease of 2-*O*-linked Rha*p* and 2,4-di-*O*-substituted Rha*p* and, for GL-PII, a decrease in non-reducing-end Gal*p*; for GL-PIII, a decrease in non-reducing-end Ara*f*, Ara*p*, Rha*p*, and Gal*p*; and for GL-PIV, a decrease in 3-*O*-linked Gal*p* and 6-*O*-linked Gal*p* in addition to those decreased in GL-PIII. These results indicated that some 2-*O*-linked Rha*p* (56% in GL-PI, 66% in GL-PII, 39% in GL-PIII, and 46% in GL-PIV) and 2,4-di-*O*-substituted Rha*p* (23% in GL-PI, 34% in GL-PII, 17% in GL-PIII, and 65% in GL-PIV) were attached to C-4 of GalA in these polysaccharides. Analysis of R_2-a from GL-PI, II, III, and IV (Table V) gave 1,5-di-*O*-acetyl-6-*O*-ethyl-2,3,4-tri-*O*-methylgalactitol, whereas that of R_2-b from GL-PII gave a trace 1,5-di-*O*-acetyl-2-*O*-ethyl-3,4-di-*O*-methylrhamnitol in addition to 1,5-di-*O*-acetyl-6-*O*-ethyl-2,3,4-tri-*O*-methylgalactitol as ethylated sugars. These results suggested that 4-*O*-linked GalA was attached to position 6 of Gal*p* (**1**) in each of the polysaccharides, and that 4-*O*-linked GalA was also linked to position

TABLE III

METHYLATION ANALYSIS OF GL-PI-GL-PIV

Glycose	*Position of O-methyl groups*	*Deduced glycosidic linkages*	*Composition (mol. %)*			
			I	*II*	*III*	*IV*
Ara	2,3,5	terminal (furanose)			1.1	2.5
	2,3,4	terminal (pyranose)			1.5	2.4
Fuc	2,3,4	terminal			1.8	
	2,3	4			1.1	
	2	3,4			2.8	2.7
	3	2,4			1.8	
Rha	2,3,4	terminal	1.9	1.4	2.6	1.4
	3,4	2	25.0	22.2	9.9	2.6
	2,4	3			1.9	
	3	2,4	5.9	8.7	3.0	4.2
Gal	2,3,4,6	terminal	4.9	7.1	5.3	7.4
	2,3,4	6	5.5	10.4	2.5	9.7
	2,3,6	4	3.6	4.5	2.8	2.0
	2,4,6	3	1.2	1.9		4.3
	2,3	4,6	2.0	1.6	1.8	1.5
	2,4	3,6	1.5	2.9	1.5	4.6
	3,6	2,4			4.1	1.2
	2	3,4,6				4.2
Glc	2,3,6	4				3.8
	2,6	3,4				2.8
GalA	2,3,4	terminal	0.6	0.5	8.7	2.4
	2,3	4	38.6	30.8	23.0	23.3
	2	3,4	2.3	1.6	6.8	2.8
	3	2,4	1.6	1.0	7.4	3.0
GlcA	2,3,4	terminal	2.1	1.7	3.3	1.0
	2,3	4	2.7	3.3		4.3
	2	3,4				3.5

2 of the 2-*O*-linked Rha*p* in GL-PII (**2**). The results also suggested that 2-*O*-linked Rha*p* in sequence **2** was linked to 4-*O*-linked GalA through some neutral sugar residue (probably 2,4-di-*O*-substituted or 2-*O*-linked Rha*p*) as the minor partial structure (**3**) in GL-PII.

DISCUSSION

The potent anti-complementary polysaccharides isolated from Ginseng leaves were pectic polysaccharides. The highly active (GL-PI, GL-PII, and GL-PIV) and the weakly active (GL-PIII) polysaccharides had different structures. GL-PI, GL-PII, and GL-PIV each consisted of a rhamnogalacturonan backbone which was substituted by neutral sugar side-chains consisting of β-D-galactan (in GL-PI and GL-PII) or arabinogalactans (in GL-PIV) attached at O-4 of Rha*p*, whereas GL-PIII consisted of a rhamnogalacturonan backbone substituted with arabino-

TABLE IV

NEUTRAL LINKAGE COMPOSITION OF GL-PI-GL-PIV BEFORE AND AFTER BASE-CATALYSED β-ELIMINATION OF URONIC ACID RESIDUES

Glycosyl residue	*Position of O-methyl groups*	*Deduced glycosidic linkages*	*Before elimination[a] (mol %)*				*After elimination[a] (mol %)*			
			I	*II*	*III*	*IV*	R_1			
							I	*II*	*III*	*IV*
Ara	2,3,5	terminal			5.5	5.1			2.3	0.6
	2,3,4	terminal			5.0	4.9			3.0	3.3
Fuc	2,3,4	terminal			5.9				6.7	
	2,3	4			3.9				4.6	
Rha	2,3,4	terminal			6.3				4.1	
	3,4	2	53.1	34.6	21.8	5.7	23.4	11.9	13.3	3.1
	2,4	3			7.4				12.6	
	3	2,4	12.4	14.1	8.7	8.8	9.6	9.3	7.0	3.1
Gal	2,3,4,6	terminal	9.8	13.6	14.7	15.3	14.9	11.6	12.6	10.7
	2,3,6	4	7.7	9.4	8.8	4.3	19.7	11.5	12.2	13.5
	2,4,6	3	2.7	3.8		9.5	8.4	11.3		7.0
	2,3,4	6	14.4	18.8	7.9	20.1	24.1	30.0	8.4	15.7
	3,6	2,4				2.4				10.2
	2,4	3,6		5.6	4.3	10.0		14.3	12.9	15.2
Glc	2,3,6	4				7.8				14.4
	2,6	3,4				5.8				3.0

[a]For the same methylated polysaccharides.

TABLE V

NEUTRAL LINKAGE COMPOSITION OF GL-PI-GL-PIV AFTER BASE-CATALYSED β-ELIMINATION OF URONIC ACID RESIDUES FOLLOWED BY ETHYLATION

Residue	*Position of O-methyl groups*	*Position of O-ethyl groups*	*Deduced linkages*	*Composition (mol %)*							
				R_2-a[a]				R_2-b[a]			
				I	*II*	*III*	*IV*	*I*	*II*	*III*	*IV*
Ara	2,3,4		terminal				1.5				
Fuc	2,3,4		terminal			6.3					
Rha	2,3,4		terminal			8.2					
	3,4		2	3.2	4.8	16.2		48.2	27.3	34.0	12.2
	3,4	2	2						trace		
	3		2,4	8.2	14.4	5.6	8.2				
Gal	2,3,4,6		terminal	20.4	11.2	15.5	11.8	17.9	16.9	21.3	24.4
	2,3,6		4	18.7	19.0	21.9	12.6	33.9	12.9	44.7	
	2,4,6		3	12.5			6.7				
	2,3,4		6	28.7	48.7	10.1	30.1		25.9		46.3
	2,3,4	6	6	8.1	1.7	9.6	4.9		16.1		
	3,6		2,4				11.5				
	2,4		3,6			6.4					17.1
Glc	2,3,6		4				12.3				

[a]From the β-elimination products in Table IV.

→4)-GalA-(1→6)-Gal-(1→ -4)-GalA-(1→2)-Rha-(1→

1 **2**

→4)-GalA-(1→2)-Rha-X-(1→4)-GalA-(1→

3

X = neutral sugar residue

galactan, highly branched Fuc chains, and GalA mostly at O-3 or O-2 of GalA. These results suggested that the branched GalA in the GL-PIII may suppress the anti-complementary activity. The rhamnogalacturonan moieties of GL-PI and II contained an alternating sequence of 2- or 2,4-di-*O*-substituted α-L-Rha*p* and 4-*O*-linked α-D-GalA, but the branching frequencies due to 2,4-di-*O*-substituted α-L-Rha*p* differed, whereas GL-PIII and IV contained a galacturonan moiety consisting of 4-*O*-linked α-D-GalA in addition to the rhamnogalacturonan moiety. GL-PII also contained a small proportion of 2-linked Rha in the backbone, and GL-PIII was rich in highly branched GalA. The β-D-galactan side-chains in GL-PI and GL-PII consisted mainly of 4-*O*-linked and 6-*O*-linked Gal*p*. Thus, GL-PI–GL-PIV each contained the possible partial structural units shown in Fig. 4.

Solov'eva and Lie *et al.* reported[36–38] structural studies of Ginseng root pectins which have anti-tumor and immunostimulating activities[37]. Only GL-PIV has a structure similar to that of these pectins.

We have reported several anti-complementary pectic polysaccharides which contain a rhamnogalacturonan moiety to which are attached β-D-galactan chains consisting of 3-, 6-, 4-, and/or 3,6-di-*O*-substituted Gal from such Chinese herbs as the roots of *Angelica acutiloba* (AGIIb-1)[11,12], the seed of *Coix-lacryma-jobi* (CA-1 and CA-2)[13], and the leaves of *Artemisia princeps* PAMP (AAFIIb-2 and AAFIIb-3)[8,14]. AGIIb-1[12], CA-1, and CA-2[13] did not contain a polygalacturonan region[8,14], whereas AAFIIb-2 and -3 were suggested to have such a region. However, another pectic polysaccharide[39] from the fruits of *Zizyphus jujuba,* which did not show potent anti-complementary activity[7], consisted[39] of a large proportion of a polygalacturonan region and a small proportion of a rhamnogalacturonan region to which were attached (1→4)-linked galactan and highly branched 3,5-arabinan as side chains. These observations suggest that the anti-complementary activity of

→2)-α-L-Rha*p*-(1→4)-α-D-GalA-(1→$_m$ →2)-α-L-Rha*p*-(1→4)-α-D-GalA-(1→$_n$ →4)-α-D-GalA-(1→$_x$ →4)-α-D-GalA-(1→$_y$

4 2 or 3

↑ ↑

SC SC

m:*n*:*x*:*y*: GL-PI = 1.0:4.2:1.3:0.7, GL-PII = 1.0:2.6:0:0.3, GL-PIII = 1.0:3.3:3.4:4.7, GL-PIV = 1.0:0.6:3.9:1.4.

Fig. 4. Possible partial structural units of GL-PI–GL-PIV; SC connotes side chains consisting of β-D-galactan (in GL-PI and GL-PII) or arabinogalactan (in GL-PIII and GL-PIV). GL-PIII is also substituted with highly branched Fuc chains and terminal GalA.

pectic polysaccharides depends on the detailed structures of the side-chain galactans and backbone rhamnogalacturonans.

The structure–activity relationship of the anti-complementary pectic polysaccharides from Ginseng leaves is being studied further.

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