ELSEVIER

Carbohydrate Research 263 (1994) 89–101

CARBOHYDRATE RESEARCH

Characterisation of an anti-ulcer pectic polysaccharide from leaves of *Panax ginseng* C.A. Meyer

Hiroaki Kiyohara, Masumi Hirano, Xiao-Guang Wen, Tsukasa Matsumoto, Xiao-Bo Sun [1], Haruki Yamada *

Oriental Medicine Research Center of the Kitasato Institute, Shirokane 5-9-1, Minato-ku, Tokyo 108, Japan

Received 28 September 1993; accepted 8 April 1994

Abstract

Structural characterisation of an anti-ulcer polysaccharide (GL-BIII), purified from leaves of *Panax ginseng* C.A. Meyer, was studied. Methylation analysis indicated that GL-BIII consisted mainly of terminal Ara*p*, 4- or 5-substituted Ara, 2,4-disubstituted Rha, 4- and 6-substituted Gal, and 3,6-disubstituted Gal. Single radial gel diffusion using β-glucosyl-Yariv antigen indicated that GL-BIII contained a small proportion of a β-(1→3,6)-galactan moiety. GL-BIII also contained terminal, 4-substituted, and 3,4-disubstituted GalA, and terminal and 4-substituted GlcA. Base-catalysed β-elimination suggested that some 2-substituted Rha in GL-BIII was attached to position 4 of a 4-substituted uronic acid. Both mild acid hydrolysis and endo-α-(1→4)-polygalacturonase digestion of GL-BIII did not give fragments consisting mainly of GalA. Methylation analysis and GC–MS analysis of acidic oligosaccharides liberated by partial acid hydrolysis indicated that GL-BIII contained a GalA-(1→4)-Rha unit in addition to longer acidic units consisting of 2-substituted Rha and 4-substituted GalA. Lithium-mediated degradation of GL-BIII followed by borohydride reduction gave small amounts of fractions containing long and intermediate neutral oligosaccharide-alditols and a large amount of a fraction containing short oligosaccharide-alditols. The long neutral oligosaccharide-alditol fraction mainly comprised 4- or 5-substituted Ara, terminal Gal*f*, 6-substituted Glc, and 2-substituted Man, whereas the intermediate oligosaccharide-alditol fraction consisted mainly of terminal and 6-substituted Gal*p*, 6-substituted Glc, and 2-substituted Man. Methylation analysis and GC–MS analysis of the short oligosaccharide-alditol fraction suggested that it contained at least

* Corresponding author.

[1] Present address: Academy of Traditional Chinese Medicine and Materia Medica of Jilin Province, Chang Chun, Jilin, China.

SSDI 0008-6215(94)00151-5

14 kinds of di- to tetra-saccharide-alditols such as Gal-(1→2)-Rha-ol, Gal-(1→4)-Rha-ol, Ara→Ara-ol, and Ara→Ara→Ara-ol.

Keywords: *Panax ginseng*; Araliaceae; Pectic polysaccharide; Structure; Anti-ulcer activity

1. Introduction

The root of *Panax ginseng* C.A. Meyer is a well known Chinese drug widely used for the treatment of gastrointestinal disorders as well as an erythropoietic and a tonic, and several pharmacologically active saponins and polysaccharides have been found in the roots of *P. ginseng* as active ingredients [1–4]. In order to investigate the clinical value of leaves of *P. ginseng*, anti-complementary and anti-ulcer activities have been compared, and a polysaccharide fraction from the leaves of *P. ginseng* was shown to be more potent for both activities than those from the roots [3,5]. Three kinds of anti-complementary pectic polysaccharides (GL-PI, PII, and PIV) have been purified [6] from the strongly acidic polysaccharide fraction of the leaves, whereas an anti-ulcer acidic polysaccharide (GL-BIII [2]) was obtained [5] from a different polysaccharide fraction of the leaves.

In the present paper, we describe structural characterisation of an anti-ulcer polysaccharide (GL-BIII) from the leaves of *P. ginseng* C.A. Meyer.

2. Experimental

Materials.—The leaves of *P. ginseng* C.A. Meyer were cultivated and collected (September, 1987) on Chang Bai mountain, Jilin, China. The acidic polysaccharide fraction (GL-4) of the leaves was prepared [5,6] by hot-water extraction, and EtOH and Cetavlon precipitations. The anti-ulcer polysaccharide (GL-BIII) was purified from GL-4 by chromatography on DEAE-Sepharose CL-6B and Sepharose CL-6B as described previously [5]. Pectinase from *Aspergillus niger* was purchased from Sigma, and endo-α-(1→4)-polygalacturonase [(1→4)-α-D-galacturonan glycanohydrolase; EC 3.2.1.15] was purified from pectinase, using the procedure [7] of Thibault and Mercier. Exo-α-L-arabinofuranosidase (EC 3.2.1.55) from *Rhodotolura flava* was a gift from Dr. N. Shibuya (National Food Research Institute, Ministry of Agriculture, Forestry and Fisheries, Japan).

General.—Carbohydrate and uronic acid in column eluates were assayed by the phenol–H_2SO_4 [8] and *m*-hydroxybiphenyl methods [9], respectively. Poly- and oligo-saccharides were hydrolysed with 2 M CF_3CO_2H at 121°C for 1.5 h, and the hydrolysates were analysed by TLC. TLC was performed on cellulose (Merck) with 5:5:1:3 EtOAc–pyridine–AcOH–H_2O as the solvent system. Reducing sugars and uronic acids were detected by alkaline $AgNO_3$ [10] and *p*-anisidine HCl [11], respectively. The sugars in the hydrolysates were converted into the corresponding alditol acetates, and analysed by GLC on a Hewlett–Packard model 5840A gas chromatograph equipped with an SP-2380 capillary column (0.2-

[2] The abbreviation of the anti-ulcer polysaccharide (GL-BIII) was changed from that (GL-4IIb$_1$-III) cited in the previous paper (Ref [5]).

μm film, 30 m × 0.25 mm i.d., Supelco). Temperature program: 60°C for 1 min, 60 → 215°C (30°C/min), 215°C for 18.8 min, 215 → 250°C (8°C/min), and 250°C for 5.7 min; the carrier gas was He (0.9 mL/min). The molar ratios of component sugars were calculated from the peak areas and response factors for the flame-ionisation detector. In order to determine the alditol components in the oligosaccharide-alditols which were liberated by degradation with lithium followed by borohydride reduction, the oligosaccharide-alditols were hydrolysed, and the hydrolysates were reduced with $NaBD_4$ and then acetylated. The resulting alditol acetates were analysed by GC–CIMS using an SP-2380 capillary column. CIMS (isobutane) was performed on a Jeol DX-300 instrument. Single radial gel diffusion using β-glucosyl-Yariv antigen was performed according to the method [12] of Holst and Clarke.

Partial acid hydrolysis of GL-BIII.—GL-BIII (10 mg) was dissolved in 10 mM CF_3CO_2H (7 mL), and heated at 100°C for 30 min. After evaporation, the products were fractionated on DEAE-Sephadex A-25 ($HCOO^-$), and four acidic fractions (A-1, A-2, A-3, and A-4) were obtained by eluting with M HCOOH, 2 M HCOOH, 5 M HCOOH, and 0.4 M NaCl, respectively, after washing with H_2O. Fraction A-4 was desalted with an electrophoretic dialyser (Microacylizer, Asahi Chemical Industry Co. Ltd., Japan) before lyophilisation.

Fraction A-2 was further hydrolysed in 0.1 M CF_3CO_2H (4 mL) at 100°C for 1 h, and the products were fractionated on DEAE-Sephadex A-25 ($HCOO^-$); the acidic fractions were obtained by eluting with 5 M HCOOH.

Degradation of GL-BIII with lithium in ethylenediamine.—GL-BIII (20 mg) was suspended in ethylenediamine (4 mL), and the solution was treated with Li according to the method of Lau et al. [13], and the reaction was stopped by addition of H_2O. The products were desalted with AG50W-X8 (H^+) resin, reduced with $NaBH_4$, and fractionated on Bio-gel P-30.

Enzymic digestion.—(*a*) *Endo-*α*-(*1 → 4*)-polygalacturonase digestion.* GL-BIII (5 mg) was stirred in 0.2 M NaOH for 2 h at room temperature, the solution was neutralised with AcOH, and the product was digested with endo-α-(1 → 4)-polygalacturonase [7] from *A. niger* in 50 mM acetate buffer (pH 4.0) at 37°C for 4 days. The products were fractionated on Bio-gel P-30 in 50 mM acetate buffer (pH 5.5).

(*b*) *Exo-*α*-*L*-arabinofuranosidase digestion.* NS-3 (7 mg) was digested with exo-α-L-arabinofuranosidase from *R. flava* in 50 mM acetate buffer (pH 4.5) at 37°C for 3 days. The products were fractionated on Bio-gel P-30.

Methylation analysis. Polysaccharides and neutral oligosaccharide-alditols were methylated once by the method [14] of Hakomori, and recovered on a Sep-pak C_{18} cartridge by the procedure [15] of Waeghe et al. except that samples were eluted only with EtOH. Uronic acids of methylated polysaccharides were reduced with $NaBD_4$ in 7:3 THF–EtOH for 18 h at room temperature followed by incubation at 80°C for 1 h, and the reduced products were recovered by desalting with AG50W-X8 (H^+) resin. Acidic oligosaccharides, which were obtained by partial acid hydrolysis, were reduced with $NaBD_4$ in M NH_4OH (4 h), and then methylated. The methylated acidic oligosaccharide-alditols were reduced with $NaBD_4$ in THF–EtOH as above, and then remethylated. The products were fractionated on Sephadex LH-20 (1:1 $CHCl_3$–MeOH) to obtain a fraction (HMW) eluted in the void volume and a lower molecular weight fraction (LMW) (detected with Molisch reagent

[16]). Each methylated sample was hydrolysed with 2 M CF_3CO_2H at 121°C for 1.5 h, and the products were converted into alditol acetates that were analysed by GLC and GLC–MS using an SP-2380 capillary column as described previously [17].

Base-catalysed β-elimination [18] *of methylated GL-BIII.*—To a solution of dry methylated GL-BIII (1 mg) in Me_2SO (1 mL) was added methylsulfinylmethanide (250 μL), and the mixture was stirred for 24 h at room temperature. To 50% of the sample was added an excess of EtI, the mixture was kept overnight at room temperature, EtI was evaporated, and the product (R_2) was recovered by using a Sep-pak C_{18} cartridge as described above. The remainder of the sample was treated with aq 50% AcOH, and the product (R_1) was obtained as described above. R_1 and R_2 each were hydrolysed, and the products were analysed as alditol acetates by GLC and GLC–MS on an SP-2380 capillary column.

GLC–MS of methylated oligosaccharide-alditols.—Solutions of methylated oligosaccharide-alditols in acetone were injected into an SP-2380 capillary column (0.2-μm film, 15 m × 0.25 mm i.d., Supelco) with on-column injection at a flow rate of 0.5 mL/min. The temperature program was 100°C for 1 min, 100 → 150°C (30°C/min), and 150 → 270°C (2°C/min). EIMS was performed on a Hewlett–Packard 5970 series mass spectrometer (70 eV, 250°C), and EIMS fragment ions [A, J, and alditol (ald)] [19] were used to determine the structures of the methylated oligosaccharide-alditols.

3. Results

Properties of GL-BIII.—GL-BIII was a homogenous polysaccharide as determined [5] by gel filtration and HPLC, and composed [5] of Rha, Ara, Man, Gal, Glc, GalA, and GlcA in molar ratios of 3:4:2:10:1:7:4. Methylation analysis suggested that it consisted mainly of terminal Ara*p* and 4- or 5-substituted Ara in addition to various linkages of Xyl, Rha, Gal, Glc, Man, GalA, and GlcA (Table 1). GL-BIII reacted with β-glucosyl-Yariv antigen weakly on single radial gel diffusion (data not shown). Digestion with endo-α-(1 → 4)-polygalacturonase gave no oligogalacturonides from GL-BIII, and the molecular weight of the products did not change (data not shown). After base-catalysed β-elimination, terminal Ara*f*, Ara*p*, Xyl, Gal, Glc, and Man, and 2-substituted Rha in GL-BIII were decreased remarkably (> 50%) (Table 1), and it was assumed that these glycosyl residues might be attached to position 4 of a uronic acid. However, in the present study, no ethylated products were detected by GC–MS in the β-elimination products.

Partial acid hydrolysis of GL-BIII.—GL-BIII was hydrolysed with 10 mM CF_3CO_2H (100°C, 30 min), the products were fractionated on DEAE-Sephadex, and four acidic fractions (A-1, A-2, A-3, and A-4) were obtained by elution with 1, 2, and 5 M HCOOH and 0.4 M NaCl, in addition to a neutral fraction. A-1, A-2, and A-3 consisted mainly of Rha, Ara, and Gal [Rha–Ara–Gal = 1.0:1.0:2.8 (A-1), 2.1:1.0:3.1 (A-2), and 0.5:1.0:2.2 (A-3) in molar ratios] in addition to GalA, GlcA, Xyl, Man, and Glc, whereas A-4 was composed mainly of Man, Gal, and Glc (Man–Gal–Glc = 1.0:2.5:1.1 in molar ratios) in addition to Rha, Ara, Xyl, and GalA. Methylation analysis suggested that A-1 and A-2 mainly contained 2,4-disubstituted Rha, 4- or 5-substituted Ara, and 6-substituted Gal; in addition, A-2 consisted mainly of 2-substituted Rha and 4-substituted GalA (Table 2). A-3 was composed mainly of 4- or 5-substituted Ara and 4-substituted Gal, whereas A-4

Table 1
Methylation analysis of anti-ulcer polysaccharide (GL-BIII) from the leaves of *Panax ginseng* C.A. Meyer

Glycosyl residue	Position of OMe group	Deduced glycosidic linkages	Mol%		
			Neutral sugar	Carboxyl-reduced	Base-catalysed β-elimination
Ara	2,3,5	terminal (furanosyl)	1.8	3.9	0.7
	2,3,4	terminal (pyranosyl)	11.0	10.0	
	2,3	4 or 5	13.5	11.0	21.0
	3	2,4 or 2,5	0.98	0.7	3.3
Xyl	2,3,4	terminal	3.9	2.5	3.5
Rha	3,4	2	3.9	4.6	
	3	2,4	7.4	7.0	4.3
Gal	2,3,4,6	terminal (pyranosyl)	6.1	6.4	2.5
	2,3,5,6	terminal (furanosyl)	2.4	2.8	1.8
	2,4,6	3	3.0	3.4	7.1
	2,3,6	4	8.6	7.6	9.1
	2,3,4	6	8.6	7.0	11.0
	2,3	4,6	3.1	1.1	
	2,4	3,6	7.4	6.3	12.0
Glc	2,3,4,6	terminal	2.3	1.2	0.6
	2,3,4	6	3.2	0.6	6.1
	2,4	3,6	0.7	0.2	2.1
	3,4	2,6	0.9	0.6	2.8
Man	2,3,4,6	terminal	1.5	1.3	0.5
GalA	2,3,4	terminal		0.3	
	2,3	4		5.0	
	2	3,4		1.3	
GlcA	2,3,4	terminal		4.1	
	2,3	4		2.3	

mainly contained 3-, 4-, and 6-substituted Gal and 4-substituted Glc. By mild acid hydrolysis, GL-BIII did not give acidic fragments consisting mainly of GalA; therefore no galacturonan moiety was contained in GL-BIII.

Because the contents of Rha and GalA in A-2 were the highest among the acidic fragments, A-2 was further partially hydrolysed with 0.1 M CF_3CO_2H (100°C, 1 h). The products were fractionated on DEAE-Sephadex, and an acidic and a neutral fraction were obtained (data not shown). Methylation analysis indicated that the high (HMW) and low molecular weight fragments (LMW) from A-2 consisted mainly of 2-substituted Rha, terminal Gal, and 4-substituted GalA (Table 3). In addition, HMW was composed mainly of 6-substituted Gal, whereas LMW mainly comprised terminal Rha and GalA. Although HMW and LMW also contained a small proportion of 3-substituted hexitol-*1-d*, the glycosyl residue could not be identified.

Permethylated carboxyl-reduced oligosaccharide-alditols in LMW were analysed by GC–EIMS, and two disaccharide-alditols (**A** and **B**) and three trisaccharide-alditols (**C**–**E**) were detected (Table 4). From the results of EIMS and methylation analysis, **A** and **C** are suggested to be GalA-(1→4)-Rha-ol-*1-d* and Rha-(1→4)-GalA→Rha-ol-*1-d*, respectively. **B**, and **D** and **E** had the structures HexA-(1→6)-Hex-ol-*1-d* and

Table 2
Methylation analysis of products from GL-BIII by partial acid hydrolysis

Glycosyl residue	Deduced glycosidic linkage	Mol%			
		A-1	A-2	A-3	A-4
Rha	terminal	trace	trace		
	2	3.3	10.7	2.8	
	4	0.9			
	2,4	11.7	10.3	7.0	
	3,4		0.7		
Ara	terminal (furanosyl)	trace	trace		
	terminal (pyranosyl)	5.4	3.3	5.2	
	4 or 5	12.4	11.0	13.8	
Xyl	terminal			4.5	
Gal	terminal (pyranosyl)	9.1	8.2	6.6	7.7
	terminal (furanosyl)			6.3	7.0
	3	4.1	3.2	5.1	13.8
	4	8.1	6.1	11.1	10.1
	6	12.6	10.5	8.1	12.4
	4,6			2.4	4.3
	3,6	7.7	5.7	6.8	5.8
	3,4,6	0.7	1.5	1.7	9.0
	2,4,6	0.5			
Glc	terminal	1.2	1.4		
	6			3.3	8.3
	4				12.7
	3,6	0.7		0.8	
GalA	terminal		1.1	2.1	
	4	9.2	15.6	5.2	9.0
	3,4	1.6	1.5	1.5	
GlcA	terminal	6.9	6.1		
	4	2.6	3.2	5.7	
	3,4	0.5			

HexA → Hex → Hex-ol-*1-d*. Although the present EIMS could not distinguish the glycosidic linkages of the hexitol-*1-d* units of **D** and **E**, the hexitol-*1-d* might be 3-substituted because 3-substituted hexitol-*1-d* was detected in the methylation analysis of LMW. Since LMW contained significant amounts of Gal, Glc, GalA, and GlcA, the glycosyl residues in **B**, **D**, and **E** could not be identified.

Analysis of neutral carbohydrate chains.—GL-BIII was subjected to Li-mediated degradation followed by borohydride reduction, and the resulting neutral oligosaccharide-alditols were fractionated on Bio-gel P-30. Small amounts of fractions (NS-1 and NS-2) eluted in the void volume and intermediate fraction and a large amount of a lowest molecular weight fraction (NS-3) were obtained (Fig. 1). NS-1 and 2 consisted of Rha, Ara, Man, Gal, and Glc in molar ratios of 0.04:0.2:1.3:1.0:0.09 and 0.05:0.3:0.7:1.0:0.1, respectively, whereas NS-3 was composed of Rha, Ara, Man, Gal, and Glc in molar ratios of 0.2:0.3:0.08:1.0:0.09. In order to analyse the alditol components of the oligosaccharide-alditols in NS-1–NS-3, each was hydrolysed, and the hydrolysates were reduced with $NaBD_4$ and acetylated. The resulting alditol acetates, which were either 1-deuterated or 1-proton-

Table 3
Methylation analysis of acidic fragments derived from A-2 [a]

Glycosyl residue	Deduced glycosidic linkage	Mol%	
		HMW	LMW
Rha	terminal	6.3	14.4
	2	11.6	13.7
	2,4	9.1	
Ara	terminal (furanosyl)	3.4	9.3
Gal	terminal	11.6	10.8
	4	6.3	4.9
	6	12.9	
	4,6	0.6	
	3,6	3.8	
Glc	terminal	2.3	4.0
	4	2.0	
Hex [b]	3 (reducing terminal)	3.9	8.8
GalA	terminal	3.7	10.3
	4	14.6	14.8
GlcA	terminal	5.6	9.0
	4	1.3	

[a] The acidic fraction (A-2) from GL-BIII was reduced with $NaBD_4$, methylated, carboxyl-reduced with $NaBD_4$, and remethylated, and then the products were fractionated on Sephadex LH-20 to obtain the high (HMW) and low molecular weight fragments (LMW).
[b] Glycosyl residue could not be deduced.

ated, were subjected to GC–CIMS. 1-Protonated rhamnitol, arabinitol, mannitol, galactitol, and glucitol were detected only in NS-3. The molar ratios of 1-protonated rhamnitol, arabinitol, mannitol, galactitol, and glucitol (as reducing terminal of oligosaccharide-alditols) were 1.3:0.3:0.1:1.0:0.05; therefore, it was suggested that most of the neutral oligosaccharides were attached to uronic acid through Rha or Gal.

Methylation analysis suggested that NS-1 consisted mainly of 4- or 5-substituted Ara, terminal Gal*f*, 6-substituted Glc, and 2-substituted Man (Table 5). NS-2 was composed mainly of terminal Gal, 6-substituted Gal and Glc, and 2-substituted Man, whereas NS-3 mainly contained 4- or 5-substituted Ara, and terminal and 6-substituted Gal. However, methylated derivatives from reducing terminals of oligosaccharide-alditols could not be detected in the present analysis.

Methylated neutral oligosaccharide-alditols from NS-3 were analysed by GC–EIMS, and 14 peaks (**a**–**n**) due to methylated di- to tetra-saccharide-alditols were detected (Fig. 2). Glycosyl units and the linkages of the oligosaccharide-alditols were deduced as shown in Tables 6 and 7. EIMS and component sugar analysis suggested that peak **a** is Rha-(1→5)-Ara-ol. Peaks **b** and **c** are believed to contain hexosyl-(1→2)-6-deoxyhexitol and hexosyl-(1→4)-6-deoxyhexitol from the results of EIMS. From the comparison of the retention time with those of standards, peak **b** is probably a mixture of Gal-(1→2)-Rha-ol and Gal-(1→4)-Rha-ol. Among the oligosaccharide-alditols peaks **e** and **l** are believed to be mono- and di-arabinosylarabinitols from EIMS and component sugar analysis. Peak **j** is proposed

Table 4
Diagnostic fragment ions on EIMS of oligosaccharide-alditols derived from acidic fragment of GL-BIII by partial acid hydrolysis

Fragment	EIMS fragment ion [*m/z* (relative abundance)]								Oligosaccharide-alditol [a]
	aJ_1	aJ_2	bA_1	bA_2	bA_3	ald			
A		206 (100)	221 (13)	189 (37)		134 (30)	309 (3)	353 (2)	GalA-(1→4)-Rha-ol-*1-d* [b]
B	296 (7)	236 (100)		189 (70)	157 (14)	146 (42)	339 (6)		HexA-(1→6)-Hex-ol-*1-d*
	aJ_2	bA_1	bA_2	abJ_1	abJ_2	cbA_1	cbA_2		
C	206 (100)	189 (91)	157 (28)	472 (91)	412 (2)	395 (2)	363 (3)		Rha-(1→4)-GalA→Rha-ol-*1-d* [c]
D	236 (100)	221 (9)	189 (52)		440 (35)				HexA→Hex→Hex-ol-*1-d*
E	236 (100)	221 (22)	189 (97)		440 (33)				HexA→Hex→Hex-ol-*1-d*

[a] HexA was detected as 6,6-dideuteriohexosyl residue.
[b] Composition of HexA in A was deduced as GalA since LMW consisted mainly of terminal GalA. Peaks A had the same retention time as GalA-(1→4)-Rha-ol although the nature of 6-deoxyhexitol-*1-d* in A could not be determined.
[c] Because LMW mainly comprised terminal Rha, GalA, and 4-substituted GalA, the glycosyl sequence of C was deduced.

to be Ara→Ara→Hex-ol by EIMS and component sugar analysis. Ara-containing oligosaccharide chains in NS-3 were digested with exo-α-L-arabinofuranosidase and the products were fractionated on the same column of Bio-gel P-30. However, pentose-containing chains in the digested products of NS-3 were mostly eluted in the same position as NS-3 (data not shown). EIMS also suggested that peaks **h** and **n** might contain di- and tri-hexosylrhamnitols, respectively. GL-BIII gave many kinds of neutral oligosaccharide-alditols (**d**, **f**, **g**–**i**, **k**, **m**, and **n**, Tables 6 and 7) containing Hex. Since NS-3 mainly comprised terminal and 6-linked Gal, many of the oligosaccharide-alditols are assumed to consist of Gal.

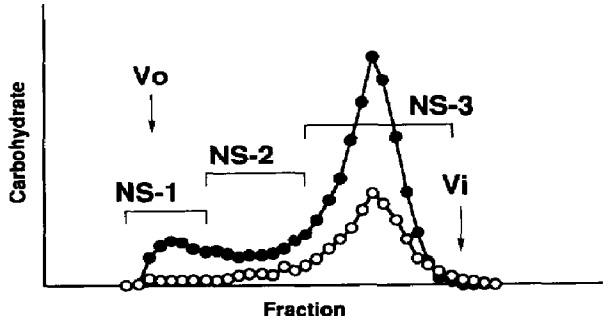


Fig. 1. Gel filtration pattern on Bio-gel P-30 of neutral carbohydrate chains derived from GL-BIII by lithium-mediated degradation: ●, carbohydrate (490 nm); ○, pentose (552 nm). Vo, void volume; Vi, inner volume.

Table 5
Methylation analysis of neutral carbohydrate chains derived from GL-BIII by lithium-mediated degradation

Glycosyl residue	Deduced glycosidic linkage	Mol%		
		NS-1	NS-2	NS-3
Ara	terminal (furanosyl)			1.9
	terminal (pyranosyl)	3.3	3.7	7.2
	4 or 5	11.8	8.5	11.2
Gal	terminal (furanosyl)	19.7	9.5	8.1
	terminal (pyranosyl)	2.7	10.2	25.7
	3			1.4
	4	3.5	2.6	7.6
	6	1.7	11.5	20.0
	4,6	2.5	3.0	2.4
	3,6		1.9	1.6
	2,4,6	0.4	0.8	
Glc	terminal	1.7	3.2	2.6
	6	10.8	13.7	2.3
	4		1.3	
Man	terminal	3.7	5.7	3.9
	2	24.2	16.5	1.3
	2,3	4.5	1.3	

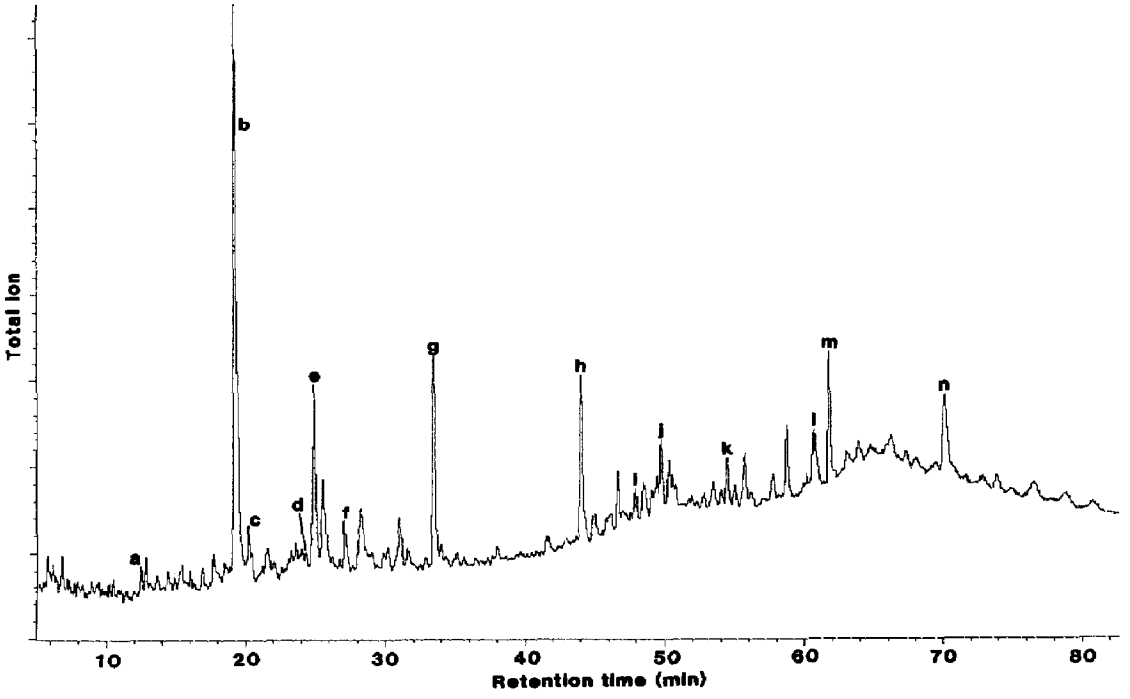


Fig. 2. Total ion chromatogram of methylated oligosaccharide-alditols derived from NS-3.

Table 6
Diagnostic fragment ions on EIMS of neutral disaccharide-alditols derived from GL-BIII by lithium-mediated degradation

Fragment	EIMS [*m/z* (relative abundance)]										Oligosaccharide-alditol
	aJ_1	aJ_2	bA_1	bA_2	bA_3	ald					
a	251 (19)	191 (100)	189 (81)	157 (22)		145 (51)					Rha-(1→5)-Ara-ol [a]
b	265 (26)	205 (100)	219 (24)	187 (71)	155 (16)	305 (2)	395 (0.2)				Gal-(1→2 and 4)-Rha-ol [b]
						133 (25)	275 (9)	307 (2)	351 (0.2)	395 (0.2)	
c	265 (5)	205 (100)	219 (21)	187 (38)	155 (0.4)	305 (4)	381 (1)				Hex-(1→2 and 4)-Rha-ol [c]
						133 (25)	275 (4)	307 (2)	381 (1)		
d	295 (4)	235 (100)	219 (23)	187 (54)	155 (11)	145 (28)	305 (3)	337 (2)	381 (3)		Hex-(1→6)-Hex-ol
e	251 (17)	191 (100)	175 (36)	143 (61)		293 (3)	305 (0.8)	337 (0.6)			Ara→Ara-ol [a]
f	295 (24)	235 (100)	219 (20)	187 (81)	155 (13)	145 (21)	349 (9)	381 (4)			Hex-(1→6)-Hex-ol
g		235 (100)	219 (13)	187 (45)	155 (11)	145 (49)	337 (5)				Hex-(1→6)-Hex-ol

[a] NS-3 consisted only of Ara and Ara-ol as pentose and pentitol.
[b] Peak b had the same retention time as GalA-(1→2)-Rha-ol and GalA-(1→4)-Rha-ol.
[c] Although deoxyhexitol unit was derived both from Rha and 3,4-disubstituted HexA [20], NS-3 contained only rhamnitol as deoxyhexitol.

4. Discussion

It has been suggested [21] that oral and subcutaneous administrations of the anti-ulcer polysaccharide (GL-BIII) inhibit the formation of gastric lesions induced by HCl-EtOH, EtOH, water immersion stress, indomethacin, or pyrolus-ligation. Recently, Yamada et al. have purified [22] the anti-ulcer pectin (Bupleuran 2IIc) from *Bupleurum falcatum* L. Bupleuran 2IIc showed anti-ulcer activity against similar gastric lesion models as GL-BIII. Bupleuran 2IIc consists [17] of a large proportion of polymerised α-(1→4)-GalA (polygalacturonan) in addition to small proportions of neutral-oligosaccharide side chains attached to a rhamnogalacturonan core (''ramified'' region) and side chains [24] [which comprised 3-deoxy-D-*manno*-2-octulosonic acid (Kdo) and α-Xyl-(1→3)-α-Fuc-(1→4)-β-Rha-(1→3′)-Apiose (Api)] attached to α-(1→4)-galacturonan. However, GL-BIII consisted mainly of neutral sugars such as Rha, Ara, and Gal in addition to small proportions of GalA and GlcA, and did not contain Kdo, 2-*O*-Me-Fuc, 2-*O*-Me-Xyl, Api, and 3-*C*-carboxyl-5-deoxy-L-xylose (aceric acid), suggesting the structure to be different from that of Bupleuran 2IIc.

In the present study, GL-BIII was subjected to base-catalysed β-elimination, sequential partial acid hydrolysis, lithium degradation, and endo-α-(1→4)-polygalacturonase and

Table 7
Diagnostic fragment ions on EIMS of neutral tri- and tetra-saccharide-alditols derived from GL-BIII by lithium-mediated degradation

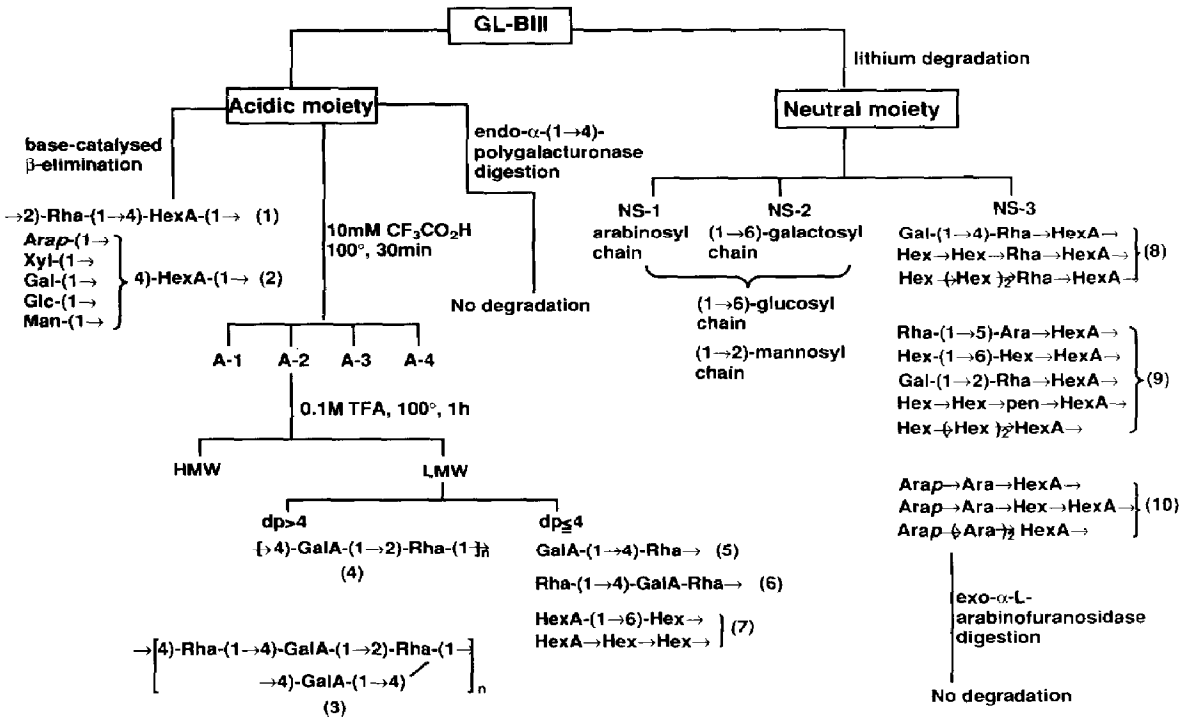
Fragment	EIMS [*m/z* (relative abundance)]									Oligosaccharide-alditol
	aJ_1	aJ_2	bA_1	bA_2	bA_3	abJ_1	abJ_2	cbA_1	cbA_2	
h	265 (23)	205 (100)	219 (33)	187 (95)	155 (33)	469 (5)	409 (3)	423 (2)	391 (0.8)	Hex → Hex → Rha-ol [a]
i		191 (100)	219 (38)	187 (63)				423 (16)	391 (2)	Hex → Hex → Pen-ol
j		235 (18)	175 (100)	143 (85)			395 (3)	335 (4)		Ara → Ara → Hex-ol [b]
k	295 (11)	235 (46)	219 (34)	187 (100)	155 (44)		439 (36)	423 (7)		Hex → Hex → Hex-ol
l	251 (4)	191 (37)	175 (74)	143 (100)	411 (8)					Ara → Ara → Ara-ol [b]
m		235 (100)	219 (19)	187 (72)	155 (20)		439 (45)			Hex → Hex → Hex-ol
	aJ_1	aJ_2	dA_1	dA_2	abJ_2	$abcJ_2$				
n	265 (12)	205 (66)	219 (34)	187 (100)	409 (2)	613 (2)				Hex → $(Hex)_2$ → Rha-ol [a]

[a] Although deoxyhexitol unit was derived both from Rha and 3,4-disubstituted HexA [20], NS-3 contained only Rha-ol as deoxyhexitol.
[b] NS-3 consisted only of Ara and Ara-ol as pentose and pentitol.

exo-α-L-arabinofuranosidase digestions, and the proposed structural units in the polysaccharide are summarised in Scheme 1.

There is a strong evidence that GL-BIII is a pectic polysaccharide. The results of mild acid hydrolysis and the endo-polygalacturonase digestion indicated that GL-BIII did not contain an α-(1 → 4)-galacturonan moiety, unlike Bupleuran 2IIc. Partial acid hydrolysis suggested that GL-BIII contained an acidic carbohydrate moiety consisting mainly of 2-substituted Rha and 4-substituted GalA. Base-catalysed β-elimination and partial acid hydrolysis indicated that GL-BIII contained glycosyl sequences as in **1** and **4**, and indicated that GL-BIII was composed of a rhamnogalacturonan core. The sequence **1**, **4**, and **5** suggested that some 2,4-disubstituted Rha of the rhamnogalacturonan cores possessed GalA side chains at position 4 of the Rha (**3**) as proposed previously [17,23,25,26]. Base-catalysed β-elimination indicated that GL-BIII contained **2**, and that the terminal glycosyl residues were attached to rhamnogalacturonan core through GalA. Methylation analysis suggested that GL-BIII contained a 3,4-disubstituted GalA moiety; however, the present results could not clarify whether the branched GalA was located in the rhamnogalacturonan core or in the side chains.

Lithium degradation suggested that at least GL-BIII contained long and intermediate size chains and 14 kinds of neutral di- to tetra-saccharides as neutral carbohydrate fragments. Partial acid hydrolysis indicated that some hexosyl chains also possessed uronic acid at the nonreducing terminals. The long and intermediate size neutral chains were found to contain



Scheme 1. Degradation procedure of GL-BIII and proposed glycosyl sequences in GL-BIII. Glycosyl sequences were proposed from structures of oligosaccharide-alditols derived by the degradations.

arabinosyl, (1→6)-substituted glucosyl, (1→6)-substituted galactosyl, and (1→2)-substituted mannosyl chains. Although GL-BIII reacted weakly with β-glucosyl-Yariv antigen, a long β-(1→3,6)-galactan might not be contained in the polysaccharide since NS-1 and NS-2 contained little or no 3,6-disubstituted Gal.

Because of the presence of **8**, it is suggested that mono- to tri-hexosyl chains are attached directly to the rhamnogalacturonan core as side chains. GL-BIII also contained **9**; therefore, it is proposed that some of the neutral carbohydrate chains are attached to the rhamnogalacturonan core through 4-linked GalA as suggested above.

The total structure of GL-BIII and the relationship between structure and anti-ulcer activity of GL-BIII must await further study.

Acknowledgement

We thank Dr. Naoto Shibuya for his kind gift of exo-α-L-arabinofuranosidase.

References

[1] R.T. Owen, *Drug Today*, 17 (1981) 343–351.
[2] C. Konno, K. Sugiyama, M. Kano, M. Takahashi, and H. Hikino, *Planta Med.*, 50 (1984) 434–436.

[3] Q.-P. Gao, H. Kiyohara, J.-C. Cyong, and H. Yamada, *Planta Med.*, 55 (1989) 9–12.
[4] X.-B. Sun, T. Matsumoto, H. Kiyohara, M. Hirano, and H. Yamada, *J. Ethnopharmacol.*, 31 (1991) 101–107.
[5] X.-B. Sun, T. Matsumoto, and H. Yamada, *Planta Med.*, 58 (1992) 445–448.
[6] Q.-P. Gao, H. Kiyohara, J.-C. Cyong, and H. Yamada, *Carbohydr. Res.*, 181 (1988) 175–187.
[7] J.-F. Thibault and C. Mercier, *J. Solid-Phase Biochem.*, 2 (1977) 295–304.
[8] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.
[9] N. Blumenkrantz and G. Asboe-Hansen, *Anal. Biochem.*, 54 (1973) 484–489.
[10] W.E. Trevelyan, D.P. Procter, and J.S. Harrison, *Nature (London)*, 166 (1950) 444–445.
[11] L. Hough, J.K.N. Jones, and W.H. Wadmann, *J. Chem. Soc.*, (1950) 1702–1706.
[12] G.-J. Holst and A.E. Clarke, *Anal. Biochem.*, 148 (1985) 446–450.
[13] J.M. Lau, M. McNeil, A.G. Darvill, and P. Albersheim, *Carbohydr. Res.*, 168 (1987) 245–274.
[14] S. Hakomori, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
[15] T.J. Waeghe, A.G. Darvill, M. McNeil, and P. Albersheim, *Carbohydr. Res.*, 123 (1983) 281–304.
[16] Z. Dische, *Methods Carbohydr. Chem.*, 1 (1962) 478–481.
[17] H. Yamada, M. Hirano, and H. Kiyohara, *Carbohydr. Res.*, 219 (1991) 173–192.
[18] M. McNeil, A.G. Darvill, and P. Albersheim, *Plant Physiol.*, 66 (1980) 1128–1134.
[19] N.K. Kochetkov and O.S. Chizhov, *Adv. Carbohydr. Chem.*, 21 (1966) 39–93.
[20] J.M. Lau, M. McNeil, A.G. Darvill, and P. Albersheim, *Carbohydr. Res.*, 168 (1987) 219–243.
[21] X.-B. Sun, T. Matsumoto, and H. Yamada, *Planta Med.*, 58 (1992) 432–435.
[22] H. Yamada, X.-B. Sun, T. Matsumoto, K.-S. Ra, M. Hirano, and H. Kiyohara, *Planta Med.*, 57 (1991) 555–559.
[23] H. Yamada, H. Kiyohara, and Y. Otsuka, *Carbohydr. Res.*, 170 (1987) 181–191.
[24] M. Hirano, H. Kiyohara, T. Matsumoto, and H. Yamada, *Carbohydr. Res.*, 251 (1994) 145–162.
[25] H. Kiyohara and H. Yamada, *Carbohydr. Res.*, 193 (1989) 173–192.
[26] H. Kiyohara and H. Yamada, *Kitasato Arch. Exp. Med.*, 64 (1991) 1–11.