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FURTHER STRUCTURAL STUDIES OF AN ANTI-COMPLEMENTARY ACIDIC HETEROGLYCAN FROM THE LEAVES OF *Artemisia princeps*

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ABSTRACT

Partial acid hydrolysis of the anti-complementary acidic heteroglycan, AAFIIb-3, isolated from the leaves of *Artemisia princeps* PAMP gave the oligosaccharides Gal-(1→6)-Gal, Gal-(1→6)-Gal-(1→6)-Gal, GalA-(1→4)-Rha, GalA-(1→2)-Rha, GlcA-(1→4)-Gal, GlcA-(1→4)-Rha, GlcA-(1→6)-Gal, and GlcA-(1→4)-Xyl. On methylation of AAFIIb-3 without de-esterification, 4-linked and 3,6-disubstituted galactan, 3-linked galactan, 4-linked galactan, and branched arabinan-rich fragments were obtained. The results of base-catalysed β-elimination indicated that AAFIIb-3 has a backbone consisting of 4-linked GalA and 2-linked Rha to which a highly branched arabino-3,6-galactan and arabino-4-galactan are linked at positions 4 of some 2-linked Rha units. Xyl-(1→4)-GalA, GlcA-(1→4)-Xyl-GalA, and →3)-Gal-(1→4)-GalA might also be joined to other 2-linked Rha at the same position. Some 6-linked and 4-linked Gal were terminated by GlcA.

INTRODUCTION

Artemisiae Argyi Folium, the leaves of *A. princeps* PAMP (Japanese name Gaiyo), is a well known Chinese drug used clinically, from which we have purified anti-complementary polysaccharides[1], AAFIIb-2 and IIb-3. AAFIIb-2 and IIb-3 (refs. 1,2) consisted of Rha, Xyl, Ara, Gal, Glc, GalA, and GlcA in the molar ratios 7.6:7.6:13.0:10.9:3.0:51.2:6.7 and 3.9:2.6:24.7:19.7:2.6:15.0:31.5, respectively. The results of carboxyl reduction, methylation analysis, and enzymic hydrolysis suggested[2] that each glycan contained a main chain consisting of (1→4)-linked GalA and (1→2)-linked Rha mostly substituted at position 4. Each glycan[2] also contained an arabino-3,6-galactan moiety, and most of the arabinose was present as α-L-furanosyl residues at the non-reducing termini and highly branched side-chains which were attached mostly to positions 3 of (1→6)-linked Gal*p*. Some non-reducing termini[2] were occupied by GlcA.

We now report further on the structure of the major polysaccharide, AAFIIb-3.

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EXPERIMENTAL

Materials and methods. — The leaves of *A. princeps* PAMP were purchased from Uchida Wakanyaku Co. Ltd. (Tokyo). Bio-gel P-2 (200–400 mesh) was obtained from Bio-Rad, QAE-Sephadex A-25, Sephadex G-15, and LH-20 from Pharmacia, and Sep-Pak C_{18} cartridges from Waters Associates Inc. Exo-α-L-arabinofuranosidase[3] from *Rhodotorula flava* was a gift from Dr. Naoto Shibuya (National Food Research Institute, Ministry of Agriculture, Forestry and Fisheries, Japan).

The carbohydrate and uronic acid in column fractions were monitored by the phenol–sulphuric acid[4] and the *m*-hydroxybiphenyl[5] methods, respectively. Component sugars of oligosaccharides were analysed[6] as the corresponding alditol acetates by g.l.c. T.l.c. of acid hydrolysates was performed on cellulose-coated plastic sheets (Merck), using ethyl acetate–pyridine–acetic acid–water (5:5:1:3). P.c. was performed on No. 51 paper (Toyo-Roshi Co. Ltd., Japan), using ethyl acetate–formic acid–acetic acid–water (18:1:3:4). Reducing sugars were detected with alkaline silver nitrate[7] and uronic acid with *p*-anisidine hydrochloride[8].

The anti-complementary polysaccharide, AAFIIb-3, isolated from the leaves of *A. princeps* PAMP and purified[1], was shown to be homogeneous by electrophoresis, ion-exchange chromatography, and gel filtration, and was digested[2] by exo-α-L-arabinofuranosidase.

Determination of carboxyl methyl groups[9]. — A solution of AAFIIb-3 (2 mg) in 0.5M sodium hydroxide (0.1 mL) containing ethanol as internal standard was stored for 1 h at 20° and then subjected directly to g.l.c. using a glass column (0.3 × 200 cm) of Gaskuropack 54 (Gasukuro Kogyo, Japan) at 120°.

Methylation analyses. — (*a*) AAFIIb-3 (3 mg) was de-esterified[10] at pH 12 for 2 h at 4° and then methylated once by the Hakomori procedure[11] in order to prevent β-elimination, but methylsulphinylcarbanion was added two or three times until the conversion of the polysaccharide into a polyalkoxide was complete as checked by using triphenylmethane[12]. The methylated polysaccharide was purified on a Sep-pak C_{18} cartridge[13] followed by gel filtration[14] on Sephadex LH-20.

(*b*) AAFIIb-3 was methylated, without de-esterification, by the above method[11], but methylsulphinylcarbanion was added only once. The reaction mixture was dialysed against water. The fully methylated oligosaccharide fractions, DF-1 and DF-2, in the diffusible fractions were eluted from a Sep-pak C_{18} cartridge with acetonitrile–water (1:4) and acetonitrile–ethanol (1:1), respectively. Extraction of the non-diffusible fraction with chloroform gave Ext-1. The water-soluble fraction was remethylated and extracted with chloroform to give Ext-2 and the process was repeated to give Ext-3.

(*c*) Each neutral and acidic oligosaccharide was reduced with sodium borodeuteride and then methylated. The methylated acidic oligosaccharide-alditols were carboxyl-reduced[2,13] with sodium borodeuteride in tetrahydrofuran–ethanol (7:3) and then methylated.

Partial acid hydrolysis of AAFIIb-3. — α-L-Arabinofuranosidase-treated

AAFIIb-3 (Af-AAFIIb-3, 45 mg) was hydrolysed with 0.25M sulphuric acid (4.5 mL) for 1.5 h at 100°. The hydrolysate was neutralised ($BaCO_3$) and then applied to a column (2 × 15 cm) of QAE-Sephadex A-25 (HCO_3^- form). The neutral fraction was eluted with water, and the acidic fraction with 0.1, 0.3, and 0.5M NH_4HCO_3. The neutral fraction was further fractionated by elution from a column (2.5 × 50 cm) of Bio-gel P-2 at 55° with water to give N-1 and N-2, eluted in the tri- and di-saccharide regions, and a monosaccharide fraction. The acidic fraction (0.1M NH_4HCO_3 eluate) was fractionated on a column (0.8 × 42 cm) of Sephadex G-15 equilibrated with water, to give Fr-1 and Fr-2 (major). Fr-2 was further purified by p.c. to give A-1 (R_{GlcA} 0.98–0.85) and A-2 (R_{GlcA} 0.83–0.61).

G.l.c-m.s. — A solution of each methylated oligosaccharide-alditol in acetone was injected into a SPB-5 capillary column (0.25-μm film thickness, 25 m × 0.25 mm i.d., SUPELCO) in the splitless mode. The temperature program was 120° for 3 min, →190° at 30°/min, and →310° at 4°/min. Both e.i. (70 eV; ionisation current, 300 μA) and c.i. (isobutane, 250 eV; accelerating voltage, 3 kv) mass spectra were recorded with a JEOL DX-300 mass spectrometer. C.i.[15] and e.i. fragment ions A, J, and alditol (ald)[16] were used to determine the structures.

β-Elimination[17,18]. — A solution of dry, methylated, de-esterified AAFIIb-3 (2 mg) in methyl sulphoxide (1 mL) was stirred with 2M methylsulphinylcarbanion (250 μL) for 24 h at room temperature. To 70% of the sample was added excess of ethyl iodide, the mixture was kept overnight at room temperature, and the partially ethylated sample was recovered[13,14] using a Sep-pak C_{18} cartridge and fractionated[14] on a column (1.0 × 25 cm) of Sephadex LH-20 to give material of high (R_2-a) and low (R_2-b) molecular weight. The remainder of the sample was neutralised with aqueous 50% AcOH, and the product (R_1) was obtained as described above. R_1, R_2-a, and R_2-b were each hydrolysed and the products were converted[14] into the alditol acetates. The linkage composition of the sample was then determined by g.l.c. and g.l.c.-m.s., using a JEOL DX-300 instrument equipped with an SPB-5 capillary column[14].

RESULTS AND DISCUSSION

AAFIIb-3 (neutral component sugars: Rha:Ara:Xyl:Gal molar ratios, 1.0:3.1:0.6:3.1* was treated with exo-α-L-arabinofuranosidase, and the undigested polysaccharide (Af-AAFIIb-3), recovered by gel filtration on Bio-gel P-2, was composed mainly of Rha, Ara, Xyl, and Gal in the molar ratios 1.0:0.8:0.5:2.9 together with large proportions of GalA and GlcA. Hydrolysis of Af-AAFIIb-3 with 0.25M sulphuric acid for 1.5 h at 100° gave neutral mono-, tri- (N-1), and di-saccharide (N-2) fractions, and the acidic mono- and oligo-saccharide (A-1 and A-2)

* Different from the preparation reported[1] previously. AAFIIb-2 and AAFIIb-3 were structurally related polysaccharides, which were eluted[1] as adjacent peaks on gel filtration and were slightly cross-contaminated.

TABLE I

DIAGNOSTIC IONS OBTAINED ON C.I.-M.S. OF METHYLATED OLIGOSACCHARIDE-ALDITOLS

Oligosaccharide fraction	*Fragment*	*Retention time[a] (min)*	*Oligosaccharide*	*Chemical-ionisation mass-spectral fragment ions* [m/z] *(relative abundance)*]				
				$(M+H)^+$	A^+	AOH_2^+	G^+	$(G-MeOH)^+$
N-2	a		hexosyl-hexitol	472 (2.0)	236 (55.6)	254 (9.4)	219 (27.8)	187 (100)
	b		hexosyl-hexitol	472 (2.8)	236 (60.8)	254 (11.3)	219 (30.5)	187 (100)
A-1	c	11.8	hexuronosyl-deoxyhexitol	444 (0.5)	206 (25.3)	224 (32.1)	221 (9.1)	189 (100)
	d	13.2	hexuronosyl-hexitol	474 (0.6)	236 (26.9)	254 (75.5)	221 (13.8)	189 (100)
A-2	e	10.6	hexuronosylpentitol	430 (0.9)		210 (73.6)	221 (29.5)	189 (100)
	f	11.2	hexuronosyl-deoxyhexitol	444 (0.3)	206 (22.7)	224 (34.8)	221 (10.8)	189 (100)
	g	11.8	hexuronosyl-deoxyhexitol	444 (1.0)	206 (74.3)	224 (7.3)	221 (38.3)	189 (100)
	h	13.2	hexuronosyl-hexitol	474 (0.6)	236 (24.5)	254 (73.8)	221 (13.8)	189 (100)
	i	16.2	hexuronosyl-hexitol	474 (3.4)	236 (60.3)	254 (45.6)	221 (10.5)	189 (100)

[a] DB-5 (SUPELCO, 0.25 mm × 25 m; He, 0.9 mL/min; 180→310° at 4°/min). Glc is eluted faster than Gal on DB-5

TABLE II

DIAGNOSTIC IONS OBTAINED ON E.I.-M.S. OF METHYLATED DISACCHARIDE-ALDITOLS

Oligosaccharide fraction	*Fragment*	*Oligosaccharide*	*E.i. mass-spectral fragment ions* [m/z] (*relative abundance*)]									
			aJ₁	*aJ₂*	*bA₁*	*bA₂*	*ald*					
N-2	a	Hex-(1→6)-Hex-ol	296 (0.3)	236 (87.1)	219 (17.6)	187 (54.8)	337 (26.1)	305 (8.4)	293 (0.7)	178 (19.2)	146 (42.9)	
	b[a]	Gal-(1→6)-Gal-ol	296 (0.3)	236 (100)	219 (15.8)	187 (51.9)	337 (29.2)	305 (0.6)	293 (0.2)	178 (11.9)	146 (37.1)	
A-1	c[a]	GalA-(1→2 and 4)-Rha-ol		206 (100)	221 (13.6)	189 (42.0)	340 (0.9)	308 (1.6)	103 (45.0)	309 (2.3)	277 (3.8)	134 (88.7)
	d	GlcA-(1→4)-Gal-ol[c]	296 (4.0)	236 (22.6)	221 (10.5)	189 (68.3)						
A-2	e[a]	GlcA-(1→4)-Xyl-ol	252 (4.6)	192 (56.2)	221 (4.7)	189 (38.6)	295 (0.2)					
	f	GlcA-(1→4)-Rha-ol	266 (3.5)	206 (32.3)	221 (11.6)	189 (84.2)	353 (0.2)	321 (0.5)	277 (0.8)	134 (13.8)		
	g[a]	GalA-(1→2 and 4)-Rha-ol		206 (100)	221 (14.9)	189 (43.8)	340 (0.3)	308 (0.6)	103 (16.4)	309 (1.0)	277 (1.8)	134 (34.0)
	h[b]	GlcA-(1→4)-Gal-ol[c]	296 (4.0)	236 (27.5)	221 (14.9)	189 (96.9)						
	i[b]	GlcA-(1→6)-Gal-ol		236 (71.1)	221 (8.2)	189 (66.2)	339 (2.7)	307 (0.3)	295 (0.3)	178 (17.1)	146 (43.9)	

[a] Major oligosaccharides. [b] Oligosaccharides in moderate amounts. [c] Assigned on the basis of g.l.c.–e.i.-m.s. and methylation analysis data.

fractions together with highly polymeric material. The neutral monosaccharide fraction contained Rha, Ara, Xyl, and Gal in the molar ratios 1.0:4.5:4.4:7.2, and the acidic monosaccharide fraction contained only GlcA.

Characterisation of the neutral oligosaccharides. — N-1 and N-2, each of which consisted of Gal and a trace of Glc, were reduced with sodium borodeuteride and then methylated. G.l.c.–c.i.–m.s. of the products from N-2 revealed two hexose disaccharide-alditol derivatives [a and b (major) in Table I]. E.i.–m.s. of *a* and *b* gave, *inter alia,* fragment ions bA_1 at *m/z* 219 and aJ_1 *m/z* 296 (Table II). These results agreed well with those of c.i.-m.s. The formation of fragment ions of the *ald* series at *m/z* 337, 293, and 178 from *a* and *b* suggested that each was a Hex-(1→6)-Hex-ol. Therefore, the major product *b* was identified as Gal-(1→6)-Gal-ol, whereas *a* was thought to contain Glc or Glc and Gal. E.i.–m.s. (Table III) of the methylated oligosaccharide-alditol from N-1 gave specific fragment ions at *m/z* 219 (cA_1), 440 (abJ_2), and 236 (aJ_2), indicatng it to be derived from a trisaccharide composed of hexose. Methylation analysis (Table IV) of N-1 indicated it to be

TABLE III

DIAGNOSTIC IONS OBTAINED ON E.I.-M.S. OF THE METHYLATED TRISACCHARIDE-ALDITOL DERIVED FROM N-1

Oligosaccharide	*Fragment ions* [m/z (*relative abundance*)]								
	cA_1	cA_2	aJ_1	aJo	aJ_2	abJ_1	abJ_2	*ald*	
Gal-(1→*X*)-Gal-(1→6)-Gal-ol[a]	219 (21.0)	187 (58.5)			236 (90.1)		440 (16.5)	541 (0.3)	146 (13.1)

[a] *X* = 3, 4 or 6.

TABLE IV

METHYLATION ANALYSIS DATA FOR OLIGOSACCHARIDE FRACTIONS

Methylated sugars[a]	*Mol. ratio*			*Linkages*
	N-1	*A-1*	*A-2*	
1,3,4,5-Me_4-Rha-*1-d*		0.1	trace	→2)-Rha
1,2,3,5-Me_4-Rha-*1-d*		0.06	trace	→4)-Rha
1,2,3,5-Me_4-Xyl-*1-d*			trace	→4)-Xyl
1,2,3,5,6-Me_5-Gal-*1-d*		0.1	0.1	→4)-Gal
1,2,3,4,5-Me_5-Gal-*1-d*	0.1		0.04	→6)-Gal
2,3,4,6-Me_4-Gal	1.0			Gal-(1→
2,3,4-Me_3-Gal	1.7			→6)-Gal-(1→
2,3,4,6-Me_4-Glc-*6,6-*d_2		1.0	1.0	GlcA-(1→
2,3,4,6-Me_4-Gal-*6,6-*d_2		3.7	0.3	GalA-(1→

[a] Derived from fully methylated oligosaccharide-alditols.

Gal-(1→6)-Gal-(1→6)-Gal.

Characterisation of the acidic oligosaccharides. — The acidic oligosaccharides in A-1 consisted of Rha and Gal in the molar ratio 2.8:1.0 together with GalA and GlcA, whereas those in A-2 consisted of Rha, Xyl, and Gal in the molar ratios 1.0:2.0:2.1 together with GalA and GlcA. The oligosaccharide-alditols derived from A-1 and A-2 were methylated, then carboxyl-reduced with sodium borodeuteride, methylated, and analysed by g.l.c.-m.s. (Tables I and II). C.i.- and e.i.-m.s. showed A-1 to contain two disaccharide fragments [*c* (major) and *d*]. Methylation analysis (Table IV) showed that A-1 contained 2- and 4-linked Rha*p*, and 4-linked Gal*p* reducing end-groups in addition to non-reducing terminal GalA and GlcA. Therefore, *c* was identified as a mixture of GalA-(1→4)-Rha-ol and GalA-(1→2)-Rha-ol, whereas *d* was thought to be HexA-(1→4)-Gal-ol. G.l.c.-m.s. showed that A-2 contained five disaccharide fragments: *e* and *g* (major), *h* and *i* (intermediate), and *f* (minor); *g* and *h* had the same retention times and same fragmentation patterns as *c* and *d*, respectively. Component *f* was identified as GlcA-(1→4)-Rha-ol because it was eluted faster than *g* in g.l.c. A-2 contained a larger amount of non-reducing terminal GlcA than GalA; therefore, *d*, *e*, and *i* were suggested to be GlcA-(1→4)-Gal-ol, GlcA-(1→4)-Xyl-ol, and GlcA-(1→6)-Gal-ol, respectively. The other oligosaccharides could not be identified because of the small quantities available.

Methylation analysis of AAFIIb-3 without de-esterification. — AAFIIb-3 contained 14.8% of methoxycarboxyl groups per total uronic acid, and methylation without de-esterification gave diffusible methylated fragments, DF-1 (short-chain oligosaccharides) and DF-2 (larger oligosaccharides) (Table V). DF-1 consisted mainly of 3-linked and 3,4,6-trisubstituted Gal*p*, whereas DF-2 consisted mainly of 4-linked Gal*p* and 2,4-disubstituted Rha together with 6- and 3-linked Gal*p*, terminal Ara*f*, and 4- or 5-linked Ara. DF-2 also contained terminal and 4-linked Xyl. The non-diffusible fragment (Ext-1) from the first methylation product consisted mainly of 4- and 3-linked Gal*p* and 3,6-disubstituted Gal*p*, whereas the non-diffusible fragment (Ext-2) from the second one consisted mainly of terminal Ara*f*, 4- or 5-linked and 3,4- or 3,5-disubstituted Ara, and 4-linked and 3,6-disubstituted Gal*p*. The non-diffusible fragment (Ext-3) from the third one consisted mainly of the same Ara residues as Ext-2, and 3-linked and 3,6-disubstituted Gal*p*. The yield of Ext-1 was about ten times higher than those of Ext-2 and Ext-3. Of the non-diffusible fragments, 2,4-disubstituted Rha was present only in Ext-1, but in small amount. However, DF-2 contained a significant amount of 2,4-disubstituted Rha. In Ext-1 and DF-1, significant amounts of terminal glycosyl residues could not be detected, possibly because of incomplete methylation or the presence of some GlcA as terminal residues. These results suggested (*a*) that base-catalysed β-elimination had occurred during the methylation of AAFIIb-3 using Hakomori base[11], without loss of the carboxyl methyl groups; and (*b*) that 3-linked galacto-oligosaccharide, various arabino-3,6-galactan-containing 4-galactans, and a highly branched Ara chain might be linked to the different 2,4-disubstituted Rha.

TABLE V

METHYLATION ANALYSIS DATA FOR THE NEUTRAL SUGAR MOIETY FROM AAFIIb-3

Glycosyl residues	*Position of O-methyl groups*	*Deduced glycosidic linkages*	*Fragments (mol %)*				
			Non-diffusible			*Diffusible*	
			Ext-1	*Ext-2*	*Ext-3*	*DF-1*	*DF-2*
Arabinosyl	2,3,5	Terminal	1.9	30.5	30.0	3.3	7.9
	2,3	4 or 5	3.4	22.5	17.2		6.8
	2	3,4 or 3,5	1.2	17.7	14.4		4.4
Rhamnosyl	3,4	2	2.7				
	3	2,4	3.8			trace	10.6
Xylosyl	2,3,4	Terminal	trace				5.7
	2,3	4	3.6				7.6
Galactosyl	2,3,4,6	Terminal	3.4	1.8	1.8	7.8	6.5
	2,3,6	4	40.5	9.5	5.4		30.3
	2,4,6	3	9.7	4.5	10.8	61.0	7.3
	2,3,4	6	4.3	4.3	6.3		8.4
	2,4	3,6	25.5	9.2	13.3		4.4
	2	3,4,6			0.9	25.0	

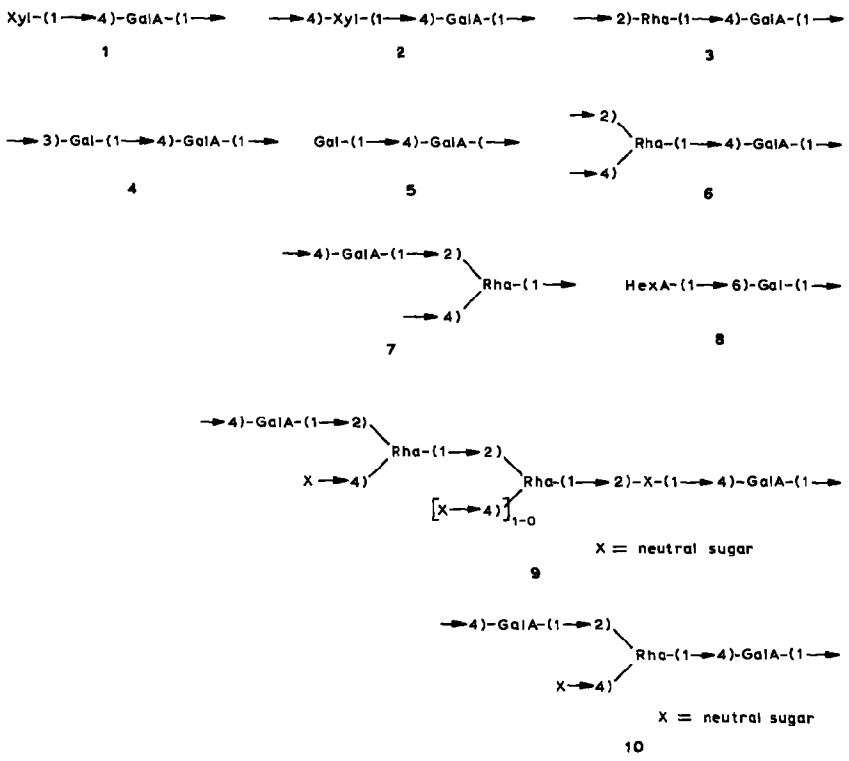
TABLE VI

LINKAGE COMPOSITION OF AAFIIb-3 BEFORE AND AFTER BASE-CATALYSED β-ELIMINATION OF URONIC ACID RESIDUES

Glycosyl residues	*Position of O-methyl groups*	*Position of O-ethyl groups*	*Deduced glycosidic linkages*	*Before elimination AAFIIb-3 (mol %)*	*After elimination*		
					R_1-HexA (mol %)	*HexA-R_2a (mol %)*	*HexA-R_2b (molar ratio)*
Arabinosyl	2,3,5		Terminal	13.7	21.3	17.5	1.0
	2,3		4 or 5	12.3	21.4	19.0	1.0
	2		3,4 or 3,5	10.5	15.4	15.5	
Rhamnosyl	3,4		2	6.6	3.0	1.3	2.0
	3		2,4	7.6	5.3	2.9	1.0
	3	2	2,4			1.5	1.0
Xylosyl	2,3,4		Terminal	4.4	1.7	1.8	1.0
	2,3		4	6.6	3.5	6.1	
Galactosyl	2,3,4,6		Terminal	6.6	4.5	3.6	2.0
	2,3,6		4	11.2	9.0	9.5	1.0
	2,4,6		3	6.9	2.4	3.4	
	2,3,4		6	7.1	7.0	8.3	1.0
	2,3,4	6	6			2.1	
	2,4		3,6	6.6	5.6	7.5	trace

Identification of rhamnogalacturonan core by base-catalysed β-elimination. — De-esterified AAFIIb-3 was methylated[11] once in order to prevent β-elimination during the methylation procedure. The base-catalysed β-elimination of methylated AAFIIb-3 was then performed, and the hydroxyl groups exposed were ethylated[17] to give products of high (R_2-a) and low molecular weight (R_2-b) isolated by gel filtration on Sephadex LH-20. Methylation analysis (Table VI, R_1-HexA) showed a decrease of terminal and 4-linked Xyl*p*, 2-linked and 2,4-disubstituted Rha*p*, terminal and 3-linked Gal*p* in AAFIIb-3 after the treatment. These results indicated that 50–65% of terminal and 4-linked Xyl*p*, 2-linked Rha*p*, and 3-linked Gal*p*, and 30% of terminal Gal*p* and 2,4-disubstituted Rha*p* were mainly attached to C-4 of Galp*A* in AAFIIb-3 (**1**–**6**). Methylation analysis of R_2-a gave 1,4,5-tri-*O*-acetyl-2-*O*-ethyl-3-*O*-methylrhamnitol and 1,5-di-*O*-acetyl-6-*O*-ethyl-2,3,4-tri-*O*-methylgalactitol, and R_2-b gave 1,4,5-tri-*O*-acetyl-2-*O*-ethyl-3-*O*-methylrhamnitol, and also terminal Ara*f*, Xyl*p*, and Gal*p*, 4- or 5-linked Ara, 2-linked and 2,4-disubstituted Rha*p*, and 4- and 6-linked Gal*p*.

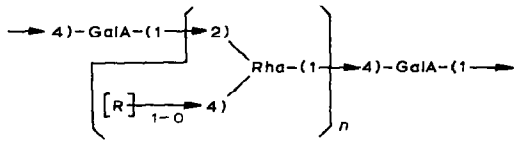
These results suggested that 4-substituted GalA was linked to position 2 of the 2,4-disubstituted Rha (**7**), and that uronic acid residues were also attached to position 6 of Gal*p* (**8**). The results of the analysis of R_2-b suggested that AAFIIb-3 possessed the partial structures shown in **9** and **10**.



DISCUSSION

The previous study[2] suggested AAFIIb-3 to be a pectic polysaccharide with a rhamnogalacturonan core and an arabinogalactan moiety attached at position 4 of Rha, as found in the rhamnogalacturonan moiety in such pectic substances[19] as rhamnogalacturonan 1 (ref. 20) (RG-1) solubilised from the cell walls of suspension-cultured sycamore cells (*Acer pseudoplatanus*). Methylation analysis of AAFIIb-3, before and after treatment with α-L-arabinofuranosidase, revealed the presence of (1→2)- and (1→2,4)-linked Rha, (1→4)-linked GalA, non-reducing terminal Ara*f*, (1→5)-linked Ara*f* or (1→4)-linked Ara, (1→3,5)-linked Ara*f*, non-reducing terminal Gal, (1→3)-, (1→4)-, (1→6)-, and (1→3,6)-linked Gal, non-reducing terminal Xyl, and GlcA. The present oligosaccharide analysis and base-catalysed β-elimination studies suggested that AAFIIb-3 contained a rhamnogalacturonan core as shown in **11**. Recently, it was shown[21] that the core structure of RG-1 contained relatively long portions of the sequence →4)-α-D-Gal*p*A-(1→2)-L-Rha-(1→. AAFIIb-3 might contain di- or tri-rhamnosyl units in the rhamnogalacturonan core, because 3,4-di-*O*-methyl-, 3-*O*-methyl-, and 2-*O*-ethyl-3-*O*-methyl-rhamnitol were detected in the small fragment produced by β-elimination. A Rha-rich region[22] has been observed in the oligosaccharides formed by acetolysis of the acidic polysaccharide from soy-bean cotyledon meal. The present results suggested that the arabino-3,6-galactan, 4-galactan, and branched arabinan might be linked to position 4 of 2,4-disubstituted Rha, and that GalA, Xyl-(1→4)-GalA, GlcA-(1→4)-Xyl-(1→4)-GalA, Gal-(1→4)-GalA, or →3)-Gal-(1→4)-GalA might be attached variously to this position. Some 6- and 4-substituted Gal*p* of arabino-3,6- or -4-galactan chains were terminated by GlcA because GlcA was present[3] only in the non-reducing terminal, and GlcA-(1→6)-Gal and GlcA-(1→4)-Gal were obtained by partial acid hydrolysis. GlcA-(1→4)-Rha was also obtained by partial acid hydrolysis of AAFIIb-3, but the location of this unit is not known.

A structure similar to that of AAFIIb-3 has been found in the acidic unit[14] of an anti-complementary pectic arabinogalactan (AGIIb-1) [refs. 14,23] isolated from the roots of *Angelica acutiloba*. AGIIb-1 also contains a dirhammnosyl unit[14] in the rhamnogalacturonan core, and GalA was attached to some Rha at position 4 as the



11

R = [R¹-(1 1-0 4)-GalA-(1→ or R²-(1→

R¹ = Xyl or GlcA-(1→4)-Xyl or →3)-Gal or Gal

R² = arabino-3,6- or -4-galactan or arabinan

side chain. The overall structure of AGIIb-1 was suggested to consist of one neutral and two acidic arabinogalactans and one neutral arabinan, which were inter-linked by acid-labile linkages. Although the structure of AAFIIb-3 was similar to that of AGIIb-1, the presence of the unit structure in AAFIIb-3 is not yet known.

The structure-activity relationships of AAFIIb-3 will be reported elsewhere.

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