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STRUCTURAL ANALYSIS OF PECTIC POLYSACCHARIDES BY BASE-CATALYSED β-ELIMINATION IN THE PRESENCE OF SODIUM BORODEUTERIDE*

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ABSTRACT

A methyl-esterified rhamnogalacturonan with neutral carbohydrate side-chains (PG-1), derived from an acidic pectic polysaccharide (AR-4IIc) from a hot-water extract of *Angelica acutiloba* Kitagawa, was subjected to base-catalysed β-elimination in the presence of sodium borodeuteride. The neutral carbohydrate chains, thereby released from position 4 of GalA residues and isolated by anion-exchange chromatography, were shown by gel-filtration chromatography and methylation analysis to consist of a high-molecular-weight galactan with some arabinosyl side-chains and material of lower molecular weight which consisted mainly of branched arabinosyl chains. Partial hydrolysis of PG-1 with acid gave a rhamnogalacturonan (MPG-1) possessing short oligosaccharide chains. β-Eliminative degradation of MPG-1 released neutral oligosaccharide chains terminated by galactitol-*1-d* and rhamnitol-*1-d* moieties. G.l.c.–m.s. showed that the neutral carbohydrates released from MPG-1 included Gal-(1→4)-Rha-ol-*1-d*, Gal-(1→4)-Gal-ol-*1-d*, Gal-(1→3)-Gal-ol-*1-d*, Gal-(1→6)-Gal-ol-*1-d*, Ara→Rha→Rha-ol-*1-d*, and Gal→Gal→Gal-ol-*1-d*.

INTRODUCTION

The acidic pectic polysaccharides[1,2] are considered to comprise a polygalacturonan region and a rhamnogalacturonan core with neutral carbohydrate side-chains ("ramified" region). The polygalacturonan region contains 4-linked α-D-GalA residues some of which are methyl-esterified. The "ramified" region contains the rhamnogalacturonan core, in which 2-linked and/or 2,4-disubstituted Rha alternate with 4-linked GalA in sequences such as →2)-Rha-(1→4)-GalA-(1→2)-Rha-(1→, and neutral carbohydrate side-chains such as arabinan, galactan, and arabinogalactan[1–3], which are assumed[1,2] to be linked to position 4 of Rha. However, the details of the linkage region between neutral carbohydrate side-chains

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and the acidic core and the heterogeneity of the neutral carbohydrate side-chains have not been clarified.

4-Linked uronic acid residues esterified with methyl[4] or vinyl groups[5] undergo β-elimination reactions[6] under alkaline[7,8] or neutral conditions[4], or on treatment with diazomethane in the presence of phosphate ion[9], with the release of the 4-substituents. The neutral carbohydrate side-chains of pectic polysaccharides are thought to be attached to position 4 of GalA through position 4 of 2-linked Rha in the acidic core[1–3] and, therefore, should be released by a β-elimination reaction. Base-catalysed β-elimination reactions have been applied to methylated bacterial[10,11] and plant polysaccharides[12,13] which contain uronic acid.

Recently, some pectic polysaccharides[14–19] possessing immunomodulating activity were obtained from plants, and side chains such as arabino-3,6-galactan were suggested to be important for the expression of complement activation[14,15,18,19].

We now report the application of the base-catalysed β-elimination reaction in the presence of sodium borodeuteride to the native pectic polysaccharide.

EXPERIMENTAL

Materials. — Crude (1→4)-α-D-polygalacturonic acid (apple) was purchased from Sigma; DEAE-Sephadex A-50 and A-25, and Sephadex G-50 and G-100 from Pharmacia; and Bio-Gel P-2 (200–400 mesh), P-4 (−400 mesh), and P-6 (−400 mesh) from Bio-Rad. The roots of *Angelica acutiloba* Kitagawa (Japanese name, Yamato Tohki) were purchased from Tochimoto Tenkaidoh Co. Ltd., and the crude pectic polysaccharide fraction (AR-4IIc)[20] was prepared by extraction with hot water, precipitation with ethanol and Cetavlon (cetyltrimethylammonium bromide), and anion-exchange chromatography. Pectinase from *Aspergillus niger* was purchased from Sigma, and endo-α-(1→4)-polygalacturonase was purified by Q.-P. Gao in our laboratory, using the procedure of Thibault *et al.*[21].

General methods. — Total carbohydrate, pentose, and uronic acid in column eluates were monitored by the phenol–sulfuric acid[22], phloroglucinol–acetic acid[23], and 3-hydroxybiphenyl[24] methods, respectively. Methyl-ester groups were assayed by the method of Wood *et al.*[25]. Glass-fibre paper electrophoresis was performed in 26mM borate buffer (pH 9.2) at 5 mA/cm for 2 h, and the polysaccharide was detected with the 1-naphthol–sulfuric acid reagent[26]. T.l.c. of acid hydrolysates was performed on cellulose, using ethyl acetate–pyridine–acetic acid–water (5:5:1:3). Neutral sugars and uronic acids were converted[27] into the alditol acetates, and analysed[15] by g.l.c. The molar ratios of uronic acid and neutral sugars in AR-4IIc were calculated from the content of uronic acid. The molar ratios of alditol acetates and alditol-*1-d* acetates were calculated from the ratios of relative intensities of $(M + H)^+$ ions of alditol acetates [*m/z* 363 (arabinitol), 377 (rhamnitol), 435 (galactitol), 364 (arabinitol-*1-d*), 378 (rhamnitol-*1-d*), and 436 (galactitol-*1-d*)] by g.l.c.–c.i.-m.s. G.l.c.–c.i.-m.s. (isobutane) was performed with a JEOL DX-300 mass spectrometer equipped with a SPB-1 capillary column (0.25-μm film thickness, 30

m × 0.25 mm i.d., SUPELCO) with the temperature programme 120 → 220° at 4°/min.

Preparation of the "ramified" and modified "ramified" regions from AR-4IIc. — AR-4IIc was de-esterified by treatment for 2 h at room temperature in 0.2M NaOH. The solution was neutralised with AcOH, and digested with endo-α-D-(1→4)-polygalacturonase[21] *(Aspergillus niger)* for 3 days at 30°. The products were fractionated on Sephadex G-50, and the fraction eluted in the void volume was the "ramified" region, PG-1. PG-1 was heated in 0.1M trifluoroacetic acid for 1 h at 100°, the solution was concentrated to dryness, and the products were fractionated on DEAE-Sephadex A-25 ($HCOO^-$ form). The acidic fraction (modified "ramified" region, MPG-1) was eluted with 5M HCOOH.

Depolymerisation of methyl-esterified (1→4)-α-D-polygalacturonic acid by β-elimination in alkaline and neutral conditions. — (1→4)-α-D-Polygalacturonic acid was suspended in freshly distilled MeOH and stirred with ethereal diazomethane[9] for 1 h at 4°. The solvents were removed in a stream of air, the methyl-esterified polygalacturonic acid was dissolved quickly in 0.1M NaOH or 20mM phosphate buffer (pH 6.8)[4], and the solution was heated for 1, 3, and 24 h at 100°. The products were fractionated on Sephadex G-50.

Depolymerisation of methyl-esterified PG-1 and MPG-1 by base-catalysed β-elimination in the presence of sodium borodeuteride. — PG-1 and MPG-1 were methyl-esterified as described above. Each product was dissolved quickly in 0.1M NaOH containing 0.2M sodium borodeuteride, and each solution was heated for 3 h at 100°, then cooled in an ice bath, and passed through a column of AG 50W-X8 (H^+) resin. The column was washed well with water, the eluate was concentrated to dryness, and boric acid was removed conventionally from each residue, using MeOH. The methyl-esterification and depolymerisation procedures were repeated at least four times, and the product was checked for residual uronic acid by t.l.c. after acid hydrolysis. The products from PG-1 and MPG-1 were each fractionated on DEAE-Sephadex A-25 ($HCOO^-$ form), and neutral (N and MN) and acidic (A and MA) fractions were obtained by eluting with water and 5M HCOOH, respectively.

Methylation analysis. — Each sample was methylated[28] and the products were purified[29] using a Sep-pak C_{18} cartridge (Waters Assoc.). Methylated MN was fractionated on Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) to give products of high (HMW) and low molecular weight (LMW). Uronic acid in the methylated products was reduced[29] with sodium borodeuteride in tetrahydrofuran–EtOH (7:3). The methylated samples were hydrolysed with 2M trifluoroacetic acid for 1.5 h at 121°, and the products were converted into the alditol acetates which were analysed[16] by g.l.c. and g.l.c.–m.s. on a SPB-1 capillary column.

G.l.c.–e.i.- and –c.i.-m.s. of methylated oligosaccharide-alditols. — This was performed as described previously[16]. C.i.[30] and e.i. fragment ions [A, J, and alditol (ald)][31] were used to determine the structures.

RESULTS AND DISCUSSION

Structure of AR-4IIc as the model pectic polysaccharide for base-catalysed β-elimination. — The acidic pectic polysaccharide AR-4IIc[20], isolated from a hot-water extract of the root of *Angelica acutiloba* Kitagawa, was eluted as a single peak from Sepharose CL-6B and gave a single spot in glass-fibre paper electrophoresis. AR-4IIc was composed mainly of Rha, Ara, Gal, and GalA in the molar ratios 1.2:3.7:1.0:4.1. Methylation analysis (Table I) revealed terminal Ara*f*, 4- or 5-linked and 3,4- or 3,5-disubstituted Ara, terminal, 4-, 3-, and 6-linked, 4,6- and 3,6-di-, and 3,4,6-tri-substituted Gal, and 2,4-disubstituted Rha in addition to a large proportion of 4-linked GalA. Methylated AR-4IIc was treated[12,13] with Hakomori base, and the products were hydrolysed, reduced, and then acetylated. G.l.c. of the resulting, partially methylated alditol acetates revealed a decrease of 2,4-disubstituted Rha (Table I). This result indicated that AR-4IIc contained the rhamnogalacturonan core, and the molar ratio of GalA and Rha also suggested the presence of the polygalacturonan region.

Base-catalysed β-elimination reaction for structural analysis of neutral carbohydrate side-chains in pectic polysaccharides. — The β-elimination reaction[6] of uronic acid moieties can occur under alkaline or neutral conditions. When the methyl-esterified (1→4)-α-D-polygalacturonic acid was heated in either 0.1M NaOH

TABLE I

METHYLATION ANALYSIS OF AR-4IIc AND THE NEUTRAL CARBOHYDRATE SIDE-CHAINS (N-1 AND N-2)

Residue	*Position of OMe groups*	*Linkages*	*Mol. %*			
			AR-4IIc Elimination		*N-1*	*N-2*
			before	*after*		
Ara	2,3,5	Terminal (furanosyl)	12.7	14.0	4.3	14.9
	2,3,4	Terminal (pyranosyl)	trace	trace	n.d.	0.5
	2,4	3 (pyranosyl)	trace	trace	0.7	4.7
	2,3	4 or 5	11.5	10.3	3.8	18.0
	2	3,4 or 3,5	12.8	11.8	1.8	14.9
	3	2,4 or 2,5	trace	trace	n.d.	1.5
Gal	2,3,4,6	Terminal	8.4	5.0	15.3	7.9
	2,3,6	4	8.8	9.7	13.0	13.0
	2,4,6	3	11.3	13.0	6.4	2.9
	2,3,4	6	17.6	17.2	21.3	6.4
	2,3	4,6	4.3	3.2	6.4	3.3
	2,4	3,2	9.1	10.8	19.5	4.8
	2	3,4,6	3.5	5.0	5.7	1.5
Rha	2,3,4	Terminal	trace	trace	n.d.	0.4
	3,4	2	trace	n.d.[a]	1.3	0.7
	3	2,4	0.3	n.d.	0.6	4.7

[a]Not detected.

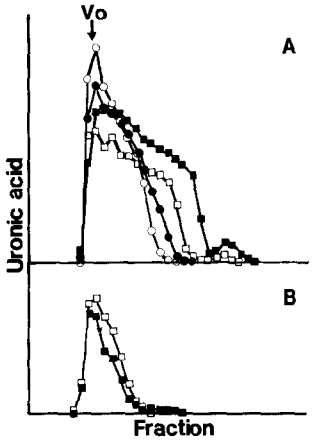


Fig. 1. Gel filtration of unesterified or methyl-esterified (1→4)-α-D-polygalacturonic acid on Sephadex G-50 after heating with *A*, 0.1M NaOH or *B*, 20mM phosphate buffer (pH 6.8): —○—, unesterified polysaccharide for 3 h at 100°; and methyl-esterified polysaccharide for 1 (—●—), 3 (—■—), and 24 h (— □—); Vo void volume; Vi, inner volume.

or 20mM phosphate buffer[4] (pH 6.8) for various times at 100° and the products were fractionated on Sephadex G-50, it was found (Fig. 1) that the degradation was more effective under the former conditions and was complete after 3 h. Therefore, treatment with 0.1M NaOH for 3 h at 100° was used in the following study.

Neutral carbohydrate chains released from position 4 of uronic acid residues by β-elimination under alkaline conditions can be degraded by "peeling" reactions[32]. β-Elimination in the presence of sodium borohydride[33] restricts the "peeling" reaction. Base-catalysed β-elimination of pectic polysaccharides in the pre-

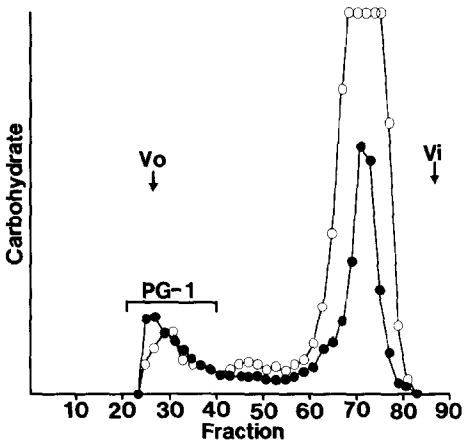


Fig. 2. Gel filtration on Sephadex G-50 of the products from AR-4IIc after digestion with endo-α-D-(1→4)-polygalacturonase: —●—, total carbohydrate (490 nm); —○—, uronic acid (520 nm).

sence of sodium borodeuteride was carried out in order to convert the reducing terminals of the carbohydrates released into 1-deuterated alditols.

Analysis of the neutral carbohydrate side-chains in the "ramified" region of AR-4IIc. — AR-4IIc was digested with endo-α-D-(1→4)-polygalacturonase[21] from *A. niger* and the products were fractionated on Sephadex G-50 (Fig. 2). A small amount of "ramified" region (PG-1) and large amounts of oligogalacturonides were obtained. PG-1 contained Rha, Ara, and Gal in the same ratios as AR-4IIc and, therefore, was used for the β-elimination reaction.

PG-1 was methyl-esterified with diazomethane and a solution in 0.1M NaOH containing 0.2M sodium borodeuteride was kept for 3 h at 100°. Elution of the products from DEAE-Sephadex A-25 ($HCOO^-$ form) with water and 5M HCOOH gave the neutral (N) and acidic (A) fractions, respectively (Fig. 3A). Since significant amounts of neutral sugars remained in fraction A, the procedure was repeated. However, a significant amount of the neutral fraction was not obtained by DEAE-Sephadex chromatography (Fig. 3B). Fraction N was composed of Rha, Ara, and Gal in the molar ratios 0.4:2.2:1.0, and fraction A consisted of Rha, Ara, and Gal in the molar ratios 2.0:0.3:1.0 in addition to GalA. When N was fractionated on Bio-Gel P-4, most of the carbohydrate chains were eluted in the void volume (data not shown). When N was further fractionated on Sephadex G-100, the fraction (N-1) eluted in the void volume and a lower-molecular-weight fraction (N-2) were obtained (Fig. 4 and Scheme 1). N-1 consisted of Ara and Gal in the molar ratio 0.1:1.0. and N-2 was composed of Rha, Ara, and Gal in the molar ratios 0.3:2.5:1.0. Methylation analysis (Table I) showed that N-1 contained mainly terminal and 4- and 6-linked and 3,6-disubstituted Gal in addition to small amounts of terminal Ara*f*, 4- or 5-linked and 3,4- or 3,5-disubstituted Ara, 2,4-disubstituted

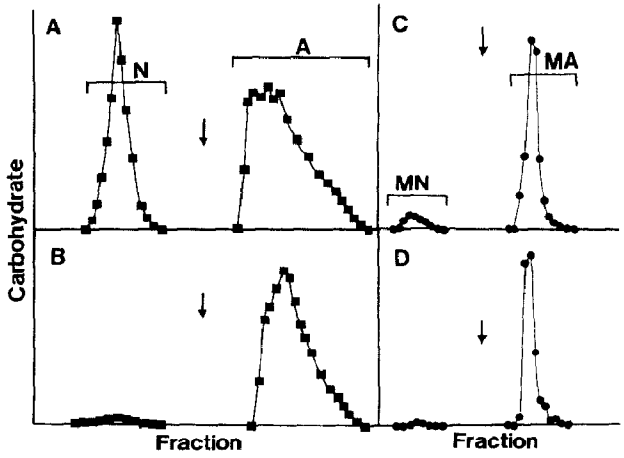


Fig. 3. Chromatography on DEAE-Sephadex of the products from the "ramified" (A and B) and modified "ramified" region (C and D) of AR-4IIc by the base-catalysed β-elimination reaction. The acidic fractions from A and C were further subjected to a β-elimination reaction, and then the products were fractionated (B and D). The acidic fractions were eluted with 5M HCOOH at the position of the arrow.

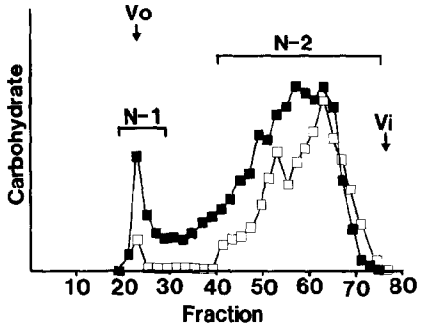
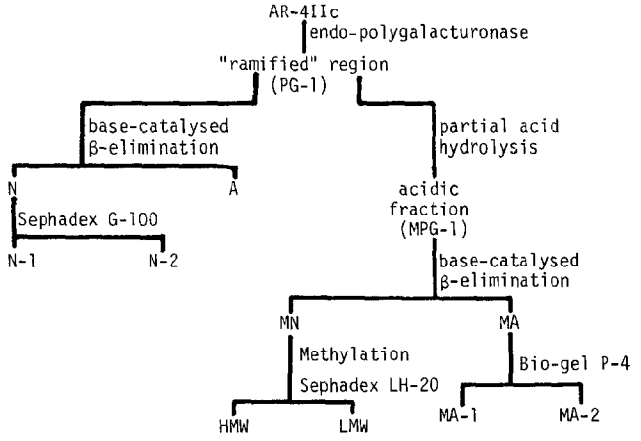


Fig. 4. Gel filtration of the neutral fraction (N) from Fig. 3A on Sephadex G-100: —■—, total carbohydrate (490 nm); —□—, pentose (552 nm).

Rha, and 3-linked, 4,6-di- and 3,4,6-tri-substituted Gal. N-2 contained a large amount of terminal Ara*f*, 4- or 5-linked and 3,4- or 3,5-disubstituted Ara, and 4-linked Gal. These results suggested that AR-4IIc contained at least two types of neutral carbohydrate side-chains, one of which was a galactan with small amounts of arabinosyl side-chains, and the other was composed mainly of branched arabinosyl chains. However, 1-deuterated alditols could not be detected in N-1 and N-2, because of their low contents in the carbohydrate chains.

Analysis of the linkage region between neutral carbohydrate side-chains and GalA in the modified "ramified" region of AR-4IIc. — When PG-1 was partially hydrolysed and the products were fractionated on DEAE-Sephadex, a large amount of neutral fraction and a small amount of acidic fraction (MPG-1) were obtained (data not shown). MPG-1 was eluted in the void volume from Bio-Gel P-6 with 50mM acetate buffer (pH 5.6), and consisted of Ara, Rha, and Gal (molar ratios 0.1:0.7:1.0) in addition to a large amount of GalA. Because MPG-1 contained 4-linked GalA and 2-linked and 2,4-disubstituted Rha in addition to terminal



Scheme 1. Isolation of the products formed from AR-4IIc by base-catalysed β-elimination reaction.

and 3-, 4-, and 6-linked and 3,6-disubstituted Gal, it was thought to consist of rhamnogalacturonan possessing short oligosaccharide side-chains. The reducing terminals in MPG-1 were reduced with sodium borohydride before the β-elimination reaction. Reduced MPG-1 was methyl-esterified and heated in 0.1M NaOH in the presence of sodium borodeuteride as described above. Elution of the products from DEAE-Sephadex with water and 5M HCOOH gave neutral (MN) and acidic (MA) fractions, respectively (Fig. 3C). When MA was treated by the above procedure, most of products were recovered as an acidic fraction (Fig. 3D). MN was composed of Rha, Ara, and Gal (molar ratios 0.2:trace:1.0), whereas MA consisted of Rha, Ara, and Gal (molar ratios 2.0:0.3:1.0) in addition to GalA. The component sugars of MN were analysed as alditol acetates by g.l.c.–c.i.-m.s., and the molar ratios of rhamnitol, rhamnitol-*1-d*, galactitol, and galactitol-*1-d* were calculated to be 1:0.6:8:2. These results suggested that 37.5% of Rha and 20% of Gal in MN were released from position 4 of GalA residues by the β-elimination reaction.

MN was methylated (Hakomori), and the products were fractionated on Sephadex LH-20 to give almost equal amounts of fractions of high (HMW) and low molecular weight (LMW) (Scheme 1). Methylation analysis (Table II) showed that HMW and LMW contained mainly terminal, 3-, 4-, and 6-linked Gal, and 4-*O*-acetyl-1,2,3,5-tetra-*O*-methylrhamnitol-*1-d* and 4-*O*-acetyl-1,2,3,5,6-penta-*O*-methyl-, 3-*O*-acetyl-1,2,4,5,6-penta-*O*-methyl-, and 6-*O*-acetyl-1,2,3,4,5-penta-*O*-methylgalactitol-*1-d* were detected in LMW. Oligosaccharide-alditols in LMW

TABLE II

METHYLATION ANALYSIS OF NEUTRAL (HMW AND LMW) AND ACIDIC (MA-1) FRACTIONS FORMED FROM THE MODIFIED "RAMIFIED" REGION (MPG-1) OF AR-4IIc BY BASE-CATALYSED β-ELIMINATION

Residue	*Position of OMe groups*	*Position of deuterium*	*Linkages*	*Mol. %*		
				HMW	*LMW*	*MA-1*
Rha	1,2,3,5	1-*d*	4 (reducing terminal)	n.d.[a]	trace	1.8
	2,3,4		Terminal	n.d.	n.d.	3.2
	3,4		2	n.d.	n.d.	13.9
	3		2,4	n.d.	n.d.	5.5
Gal	1,2,3,5,6	1-*d*	4 (reducing terminal)	n.d.	trace	n.d.
	1,2,4,5,6	1-*d*	3 (reducing terminal)	n.d.	trace	n.d.
	1,2,3,4,5	1-*d*	6 (reducing terminal)	n.d.	trace	0.8
	2,3,4,6		Terminal	29.5	58.8	18.0
	2,3,6		4	18.9	11.2	8.3
	2,4,6		3	11.5	11.8	2.8
	2,3,4		6	26.5	11.8	23.5
	2,4		3,6	6.2	n.d.	5.5
	2,3		4,6	5.3	6.5	n.d.
	2		3,4,6	2.1	n.d.	n.d.
GalA	2,3	6,6-d_2	4	n.d.	n.d.	13.9[b]

[a]Not detected. [b]Carboxyl groups of GalA residues were reduced with sodium borodeuteride after methylation of MA-1.

TABLE III

DIAGNOSTIC IONS OBTAINED ON C.I.-M.S. OF METHYLATED DISACCHARIDE-ALDITOLS OF THE NEUTRAL FRACTION FORMED FROM MPG-1 BY BASE-CATALYSED β-ELIMINATION

Alditol	*Fragment ions* [m/z *(relative abundance)*]					
	$(M+H)^+$	$(M+H)^+ -MeOH$	aJ_2	$aJ_2OH_2^+$	bA_1	bA_2
Hex→Rha-ol-*1-d*	442 (0.6)	410 (23.6)	206 (12.3)	224 (10.1)	219 (36.5)	187 (100)
Hex→Hex-ol-*1-d*	472 (2.3)	440 (1.0)	236 (33.0)	254 (50.7)	219 (100)	187 (46.5)
Hex→Hex-ol-*1-d*	472 (4.5)	440 (7.0)	236 (17.5)	254 (100)	219 (22.7)	187 (99.7)
Hex→Hex-ol-*1-d*	472 (7.0)	440 (5.1)	236 (10.8)	254 (57.6)	219 (39.1)	187 (100)

TABLE IV

DIAGNOSTIC IONS OBTAINED ON E.I.-M.S. OF METHYLATED DISACCHARIDE-ALDITOLS OF THE NEUTRAL FRACTION FORMED FROM MPG-1 BY BASE-CATALYSED β-ELIMINATION

Alditol	*Fragment ions* [m/z *(relative abundance)*]									
	aJ_1	aJ_2	bA_1	bA_2	*ald*					
Gal-(1→4)-Rha-ol-*1-d*	266 (11.8)	206 (69.4)	219 (20.5)	187 (76.7)	395 (0.1)	351 (0.1)	319 (0.2)	134 (32.6)		
Gal-(1→3)-Gal-ol-*1-d*	296 (1.8)	236 (33.3)	219 (17.4)	187 (92.0)	426 (0.4)	382 (0.6)	350 (0.4)	338 (0.5)	306 (0.5)	133 (41.0)
Gal-(1→4)-Gal-ol-*1-d*	296 (9.0)	236 (55.2)	219 (25.4)	187 (100)	381 (1.0)	349 (2.4)	305 (1.1)	134 (26.0)		
Gal-(1→6)-Gal-ol-*1-d*	296 (0.2)	236 (79.2)	219 (62.9)	187 (90.3)	337 (2.5)	305 (0.3)	293 (0.3)	249 (0.5)	178 (10.1)	146 (48.3)

TABLE V

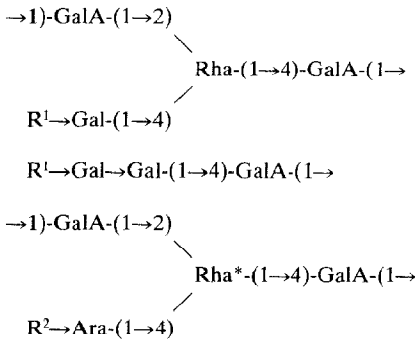
DIAGNOSTIC IONS OBTAINED ON C.I.- AND E.I.-M.S. OF METHYLATED TRISACCHARIDE-ALDITOLS OF THE NEUTRAL FRACTION FORMED FROM MPG-1 BY BASE-CATALYSED β-ELIMINATION

Alditols	*Fragment ions* [m/z *(relative abundance)*]								
	C.i.						*E.i.*		
	aJ_2	aJ_2OH_2	cA_1	cA_2	abJ_2[a]	(acJ_2+H)[b]	abJ_1	abJ_2	cbA_1
Ara→Rha→Rha-ol-*1-d*	206 (87.2)		175 (100)	143 (85.4)	380 (10.6)	398 (0.5)			
Gal→Gal→Gal-ol-*1-d*	236 (10.8)	254 (20.8)	219 (17.4)	187 (69.4)		472 (4.7)	500 (1.4)	440 (0.2)	423 (0.7)

[a]Reported by Chizhov *et al.*[30]. [b]Reported by McNeil[34].

TABLE VI

PARTIAL STRUCTURES IN THE LINKAGE REGION BETWEEN THE NEUTRAL CARBOHYDRATE SIDE-CHAINS AND THE GALACTURONIC ACID IN AR-4IIc[a]



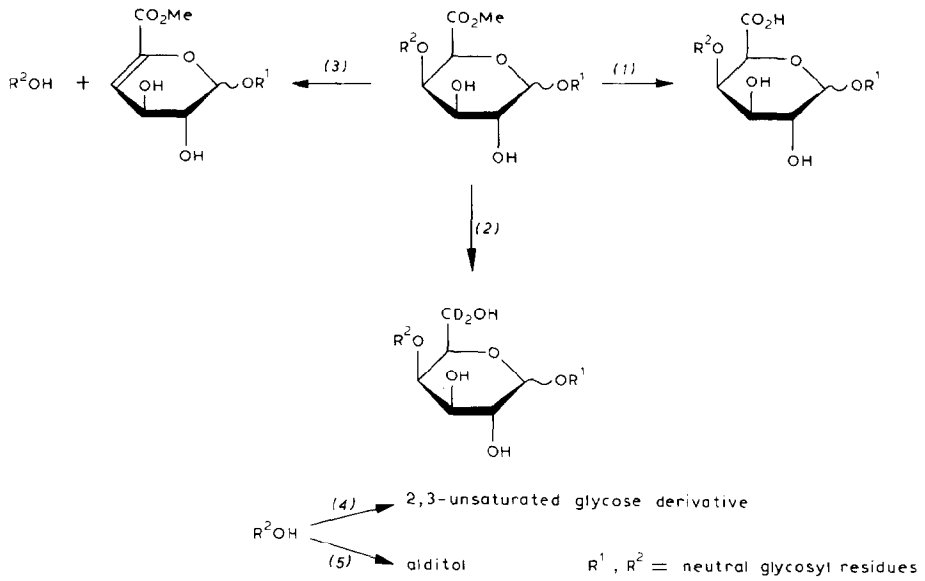
[a]R¹, galactosyl chain; R², arabinosyl chain; the arabinosyl chain is assumed to be attached to position 4 of the 2,4-disubstituted Rha marked *.

were analysed by g.l.c.–c.i.- and –e.i.-m.s. (Tables III–IV). The disaccharide-alditols (Tables III–IV) Gal-(1→4)-Rha-ol-*1-d*, Gal-(1→4)-Gal-ol-*1-d*, Gal-(1→3)-Gal-ol-*1-d*, and Gal-(1→6)-Gal-ol-*1-d*, and the trisaccharide-alditols (Table V) Ara→Rha→Rha→ol-*1-d* and Gal→Gal→Gal-ol-*1-d* were detected. These results suggested that arabinosyl chains and some galactosyl chains are attached to position 4 of GalA through position 4 of 2,4-disubstituted Rha, and that many of galactosyl chains are directly attached to position 4 of GalA in AR-4IIc as shown in Table VI.

Analysis of the acidic fraction (MA) derived from MPG-1 by β-elimination reaction. — Fractionation of MA on Bio-Gel P-4, using 50mM acetate buffer (pH 5.6), gave fractions in the void volume (MA-1) and of lower molecular weight (MA-2) (data not shown). MA-1 consisted of Rha, Ara, and Gal in the molar ratios 2.3:0.2:1.0 in addition to a significant amount of GalA, whereas MA-2 was composed mainly of GalA in addition to small amounts of neutral sugars. MA-1 was eluted from Bio-Gel P-6 in the void volume, methylation analysis (Table II) showed it to consist mainly of 2-linked Rha, terminal and 6-linked Gal, and 4-linked GalA, and 4-*O*-acetyl-1,2,3,5-tetra-*O*-methylrhamnitol-*1-d* and 6-*O*-acetyl-1,2,3,4,5-penta-*O*-methylgalactitol-*1-d* were detected. These results suggested that MA-1 consisted of at least two acidic carbohydrate chains, each of which contained 4-linked rhamnitol-*1-d* and 6-linked galactitol-*1-d* as the respective reducing terminals.

GENERAL DISCUSSION

Treatment of methyl-esterified polygalacturonic acid with alkaline sodium borodeuteride causes (*1*) saponification of the methyl-ester groups, (*2*) carboxyl-re-



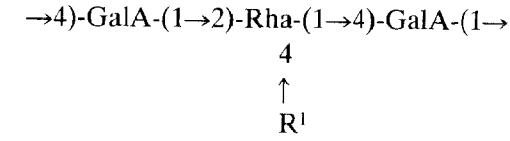
Scheme 2. Formation of derivatives during the base-catalysed β-elimination reaction in the presence of sodium borodeuteride.

duction, and (*3*) β-elimination at C-4 of methyl-esterified GalA (Scheme 2). The methyl-esterified polygalacturonic acid was degraded more under alkaline than under neutral conditions (Fig. 1), suggesting that β-elimination was fast relative to saponification. Carboxyl-reduction was slow because Gal-6,6-d_2 derivatives were not detected after application of the β-elimination reaction to AR-4IIc. The glycosyl residue (R_2OH), released from position 4 of GalA, may be converted into a 2,3-unsaturated derivative ("peeling" reaction, *4*) or an alditol (reduction, *5*) (Scheme 2). The latter reaction is thought[33] to be more rapid as shown in the β-elimination reaction of serine- and threonine-linked carbohydrate chains in glycoproteins.

The native "ramified" region of AR-4IIc was shown to comprise a high-molecular-weight galactan with some arabinosyl side-chains and a carbohydrate chain of lower molecular weight with branched arabinosyl chains which are released only from position 4 of GalA residues in the rhamnogalacturonan core by the β-elimination reaction.

Rhamnogalacturonan possessing short oligosaccharide side-chains (MPG-1), derived from the "ramified" region of AR-4IIc by partial acid hydrolysis, released di-, tri-, and oligo-saccharide-alditol-*1-d*. Because 20% of the Gal and 37.5% of the Rha in the released neutral oligosaccharide chains were present as alditol-*1-d* derivatives, these were originally linked to position 4 of GalA residues. Since the ratio of rhamnitol-*1-d* and galactitol-*1-d* was 1:4, 80% of the oligosaccharide-alditols were linked with position 4 of GalA residues in the rhamnogalacturonan core through Gal. Some pectic polysaccharides contain[16] the sequence →4)-GalA-

(1→4)-Rha-(1→, and the galactan or galactosyl side-chains in AR-4IIc may be linked to position 4 of GalA residues which are attached to rhamnogalacturonan through position 4 of 2,4-disubstituted Rha residues (**1**). Analysis of the neutral oligosaccharide-alditols, produced by the β-elimination reaction, also suggested that arabinosyl chains might be linked to the rhamnogalacturonan core only through position 4 of 2,4-disubstituted Rha residues in the core (**2**), whereas galactosyl chains might be attached to the core either through 4-linked GalA or 2,4-disubstituted Rha residues (**1** and **2**, respectively).



1 R^1 = $(Gal)_n$→Gal-(1→4)-GalA-(1→
2 R^2 = $(Ara)_n$→Ara-(1→ or
$(Gal)_n$→Gal-(1→

Lau *et al.*[35] have reported the structural analysis of Rhamnogalacturonan I (RG-I) by using lithium in ethylenediamine, and it was indicated that the neutral carbohydrate side-chains were linked to the rhamnogalacturonan core either through 2,4-disubstituted Rha residues directly or through 4-linked GalA residues, as in AR-4IIc. However, most of the neutral carbohydrate side-chains of RG-I were attached directly to 2,4-disubstituted Rha residues.

The base-catalysed β-elimination reaction of AR-4IIc left an acidic fraction which contained some neutral sugars with a large amount of GalA, and further reaction did not release neutral carbohydrate chains from the remaining acidic fraction. MA consisted of oligogalacturonides (MA-1) possessing rhamnitol-*1-d* and galactitol-*1-d* as the reducing terminals, and oligogalacturonides (MA-2). It is suggested that these reducing terminals in MA-1 were attached to MA-2 before the elimination reaction. This suggestion assumes that the neutral carbohydrate chains, which were linked to oligogalacturonide chains both at the reducing and non-reducing terminals, were present in AR-4IIc. The detailed structure of the base-catalysed β-elimination-resistant carbohydrate sequence must await further study.

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