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Pectic Substances. I. The Major Pectin from the Fruits of *Zizyphus jujuba* MILLER var. *inermis* REHD.

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The major acidic polysaccharide, named Zizyphus-pectin A, was isolated from the fruits of *Zizyphus jujuba* MILLER var. *inermis* REHD. The final preparation was homogeneous as determined by ultracentrifugal analysis, cellulose acetate and glass-fiber electrophoresis, and gel chromatography. It was composed of D-galacturonic acid, L-rhamnose, D-galactose, and L-arabinose in the molar ratio of 35 : 1 : 1 : 4, and its molecular weight was estimated to be 263000. The major part (58%) of D-galacturonic acid exists as the methyl ester. *O*-Acetyl groups were identified and the content amounted to 2.3%. Partial acid hydrolysis gave 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose, and di- to penta- saccharides consisting of α-1→4-linked D-galactopyranosyluronic acid units. In addition, reduction of carboxyl groups followed by methylation analysis showed that the pectin possesses a backbone chain consisting of α-1→4-linked D-galactopyranosyluronic acid residues which are interspersed with α-1→2-linked L-rhamnopyranose residues having side chains at position 4. The pectin has β-1→4-linked D-galactopyranosyl linear side chains and α-1→5-linked L-arabinofuranosyl highly branched side chains having 1→3-linked branching points.

Keywords——*Zizyphus jujuba* var. *inermis*; Zizyphus-pectin A; molecular weight; analysis of components; reduction and methylation; partial acid hydrolysis; NMR; rhamnogalacturonan backbone; galactan side chain; arabinan side chain

Zizyphi Fructus (Japanese name, Taisou), the fruits of *Zizyphus jujuba* MILLER var. *inermis* REHD. (Rhamnaceae), is a well-known crude drug used as an emollient, abirritant, antasthenic, and antibechic. In 1969 and 1973, Tomoda *et al.*[1,2] reported the isolation of acidic polysaccharides in addition to monosaccharides, oligosaccharides, and an arabinan from this crude drug, but the structure of the acidic polysaccharides has been left unsolved. Recently, Tomoda *et al.*[3] proposed a useful identification method for this crude drug by a cellulose acetate membrane electrophoresis of acidic polysaccharides. We have now isolated two pure acidic polysaccharides from this crude drug. The properties and the structural features of the major acidic polysaccharide are described in the present paper.

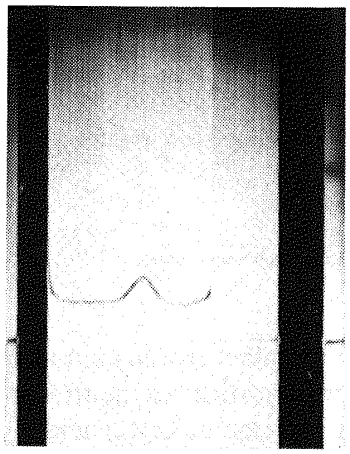

Fig. 1. Ultracentrifugal Pattern of Zizyphus-pectin A

0.5% in H_2O, 20°C, 18 min, 55430 rpm, Hitachi UCA-1A ultracentrifuge (a synthetic boundary cell).

After removal of seeds, the fruits were sliced, homogenized and extracted with hot water. The extract was applied to a column of diethylaminoethyl (DEAE)-Sephadex A-25 (acetate form). A large amount of neutral sugars was eluted with water. The major acidic polysaccharide was obtained from the eluate with 0.5 M acetate buffer (pH 5.0). In addition, a minor acidic polysaccharide was eluted with 1 M solution of the same buffer.

The major acidic polysaccharide gave a single spot on cellulose acetate and glass-fiber electrophoresis and was homogeneous as determined by ultracentrifugal analysis (Fig. 1). Furthermore, it gave a single peak on gel chromatography with Toyopearl. It showed a high positive specific rotation ($[\alpha]_D^{22}$ +201.2° in H_2O, c=0.3). Gel chromatography gave a value of 263000 for the molecular weight.

As component sugars of the polysaccharide, D-galacturonic acid, L-rhamnose, D-galactose, and L-arabinose were identified by cellulose thin-layer chromatography (TLC) of the hydrolysate and by gas-liquid chromatography (GLC) of its derivatives. These sugars were isolated by preparative paper partition chromatography (PPC) and proved to have the configurations given above. Quantitative determination of component sugars showed that the molar ratio of galacturonic acid : rhamnose : galactose : arabinose is 34.9 : 0.9 : 1.0 : 4.0.

The ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum of the polysaccharide shows signals having chemical shifts of 2.07 and 3.80 ppm. They suggest the presence of *O*-acetyl groups and *O*-methyl groups as carboxylic acid methyl esters. The presence of these groups was confirmed by GLC of the hydrolysate, and the acetyl and the methoxyl contents were determined to be 2.3 and 7.6%, respectively. Thus 58% of the carboxyl groups in the polysaccharide exist as methyl esters. The name "Zizyphus-pectin A" is prposed for this major acidic polysaccharide.

The carboxyl groups of galacturonic acid residues in the pectin were reacted with a carbodiimide reagent, then reduced with sodium borohydride to the corresponding neutral sugar units.[4] Methylations of the original and the carboxyl-reduced polysaccharides were performed with methylsulfinyl carbanion and methyl iodide in dimethyl sulfoxide.[5] The fully methylated products were hydrolyzed and the products were analyzed by gas-liquid chromatography-mass spectrometry (GLC-MS) after conversion into alditol acetates.[6] The products from the original pectin were identified as 2,3,5-tri-*O*-methyl-L-arabinose, 2,3-di-*O*-methyl-L-arabinose, 2-*O*-methyl-L-arabinose, 3,4-di-*O*-methyl-L-rhamnose, 3-*O*-methyl-L-rhamnose, 2,3,4,6-tetra-*O*-methyl-D-galactose, and 2,3,6-tri-*O*-methyl-D-galactose in a molar ratio of 3.7 : 5.2 : 3.1 : 1.0 : 1.8 : 1.2 : 1.9, while the same products from the carboxyl-reduced one were obtained in a molar ratio of 3.6 : 4.6 : 2.7 : 1.0 : 1.7 : 1.0 : 102.3.

These results suggest that the minimal repeating unit of Zizyphus-pectin A is composed of eight kinds of component sugar units as shown in Chart 1.

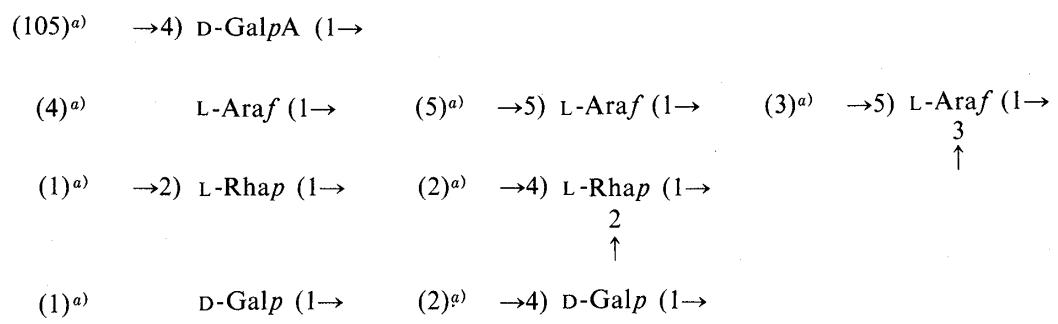

Chart 1. Component Sugar Residues in the Minimal Repeating Unit in the Structure of Zizyphus-pectin A

a) Number of residues.
Gal*p*A, galactopyranosyluronic acid; Ara*f*, arabinofuranose; Rha*p*, rhamnopyranose; Gal*p*, galactopyranose.

The high positive value of the specific rotation suggests that D-galacturonic acid residues in the pectin are α-linked.[7] For the determination of the configuration of neutral sugar linkages, the pectin was acetylated and oxidized with chromium trioxide in acetic acid. The recoveries of L-arabinose, D-galactose, and L-rhamnose were 100, 32 and 63% after 1 h, respectively. These results indicate that L-arabinose residues are α-linked, D-galactose residues β-linked, and L-rhamnose residues α-linked.[8,9] Angyal and James[8] reported that both α- and β-anomers of acetylated hexofuranosides were readily oxidized by the chromium trioxide oxidation method. However, the acetylated α-L-arabinofuranoside is resistant to the

oxidation in the same manner.[9] In order to confirm the configuration of the arabinose linkages, the pectin was digested with the α-L-arabinofuranosidase from *Rhodotorula flava*[10] and 68.1% of the arabinose was liberated after incubation for 30 min. This result provides additional evidence that the L-arabinose residues are α-linked.

On the other hand, the pectin was hydrolyzed with 0.2M trifluoroacetic acid at 80°C for 1 h. After removal of the acid, the products were applied to a column of Sephadex G-15. The high molecular weight fraction obtained was further hydrolyzed with 0.2M trifluoroacetic acid at 100°C for 5 h. After removal of the acid followed by the gel chromatography on Sephadex G-15, the intermediate molecular weight fraction obtained was finally hydrolyzed with 2M trifluoroacetic acid at 100°C for 3 h. The hydrolysate was applied to a column of DEAE-Sephadex A-25 (formate form), and L-rhamnose, D-galacturonic acid, and five oligosaccharides (I to V) were obtained from the main fractions by stepwise elution with dilute formic acid. The preparation of the partial hydrolysates is summarized in Chart 2.

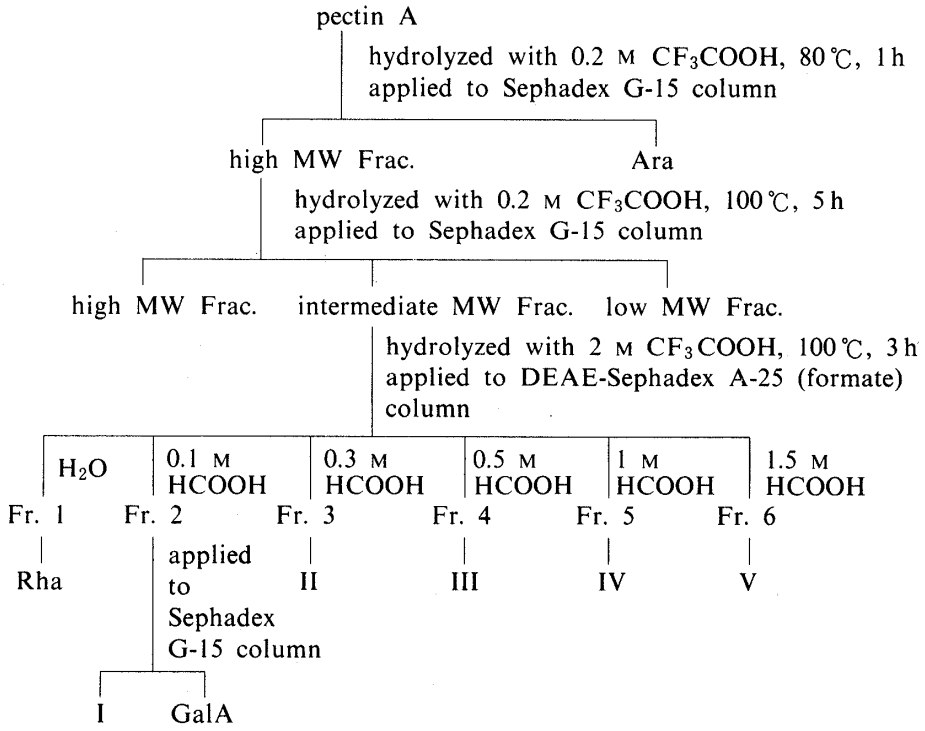

Chart 2. Isolation of the Products obtained by Partial Hydrolysis

Ara, arabinose; Rha, rhamnose; GalA, galacturonic acid.

About four-fifths of L-arabinose residues were hydrolyzed by the primary partial hydrolysis. The rest of L-arabinose and all the D-galactose residues were hydrolyzed by the secondary partial hydrolysis. Small amounts of L-rhamnose and D-galacturonic acid were also found in the low molecular weight fraction obtained by the same hydrolysis. D-Galacturonic acid was the sole component in the high molecular weight fraction obtained by the secondary partial hydrolysis. This fraction showed a high positive specific rotation ($[\alpha]_D^{22}$ $+230.0°$ in H_2O, $c=0.3$). The upward change in the optical rotation, with the concomitant removal of the neutral sugar components, supports the conclusion obtained by the chromium trioxide oxidation method regarding the configurations of glycosidic linkages.

Each of the oligosaccharides (I to V) obtained by the final partial hydrolysis was purified by chromatography on Sephadex G-15. The homogeneity of each oligosaccharide was checked by cellulose TLC. Based on the results of component sugar analysis, and by comparing its chromatographic and electrophoretic properties and the value of specific

rotation with those of an authentic sample,[11,12] 1 was identified as 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose.

Oligosaccharides II to V are composed of D-galacturonic acid alone. Based on the results of the reducing power measurement, we concluded that II, III, IV, and V are a disaccharide, a trisaccharide, a tetrasaccharide, and a pentasaccharide. As already shown in Chart 1, they must be α-1→4-linked linear oligosaccharides.

Methylation analysis of the intermediate molecular weight fraction obtained by the secondary partial hydrolysis was performed as described above, and 3,4-di-*O*-methyl-L-rhamnose was found as the sole neutral sugar methyl ether in the hydrolysis products. This result confirms the elimination of side chains in the pectin by the secondary partial hydrolysis.

Based on the accumulated evidence described above, we concluded that the main backbone chain in Zizyphus-pectin A is composed largely of α-1→4-linked D-galactopyranosyluronic acid residues, being interspersed with α-1→2-linked L-rhamnopyranose residues, and that two-thirds of L-rhamnose residues possess side chains at position 4. As already mentioned above, 58% of the D-galacturonic acid units exist as their methyl esters. There are two kinds of side chains. One of them is an α-1→5-linked L-arabinofuranosyl highly branched chain having 1→3-linked branching points. The other is composed of β-1→4-linked D-galactopyranose.

2-*O*-(α-D-Galactopyranosyluronic acid)-L-rhamnose, 4-*O*-(α-D-galactopyranosyluronic acid)-D-galacturonic acid and its homologous trisaccharide have been isolated from the partial acid hydrolysates of pectins from various sources, such as cotyledon meals and hulls of *Glycine max*,[13,14] leaves and stems of *Medicago sativa*,[15] roots of *Dianthus caryophyllus*,[16] peels of *Citrus limon*,[17] seed hulls of *Brassica campestris* subsp. *napus* var. *nippo-oleifera*,[18] and suspension-cultured cells and leaves of *Nicotiana tabacum*.[19,20] Thus pectins generally have similar back bones consisting of α-1→4-linked D-galactopyranosyluronic acid residues which are interspersed with 1→2-linked L-rhamnopyranose residues. In addition, the presence of several types of side chains, such as galactan, arabinan, xylan, or arabinogalactan, has been reported.[21,22]

The results of partial hydrolysis did not provide any evidence of the presence of arabinogalactan or galactoarabinan in Zizyphus-pectin A. Based on the result of methylation analysis of the intermediate molecular weight fraction obtained by the secondary partial acid hydrolysis, it is suggested that both linear galactan side chains and highly branched arabinan side chains are linked to the rhamnogalacturonan backbone through position 4 of the rhamnose residues in Zizyphus-pectin A.

The presence of relatively many highly branched arabinose units as neutral sugar components is characteristic of Zizyphus-pectin A, but the mode of the sequence of branches in the arabinan chains remains to be investigated. The results of detailed analysis of the structure will be reported in subsequent papers.

Experimental

Solutions were concentrated at or below 40 °C with rotary evaporators under reduced pressure. Optical rotation was measured with a JASCO DIP-SL automatic polarimeter. ^{1}H-NMR spectra were recorded on a JEOL MH-100 NMR spectrometer in heavy water containing 2,2-dimethyl-2-silapentane-5-sulfonate as an internal standard at 70 °C. GLC was carried out on a Hitachi 063 gas chromatograph equipped with a hydrogen flame ionization detector. GLC–MS was performed with a JEOL JGC-20K gas chromatograph and a JEOL JMS-D100 mass spectrometer. Infrared (IR) spectra were recorded on a JASCO IRA-2 infrared spectrophotometer.

Isolation of Polysaccharide——Chinese Zizyphi Fructus (120 g), which contained 14.6% water, were sliced and the seeds were removed. The slices were extracted twice with water (600 ml) under stirring at 90 °C for 30 min each time. The extracts were combined and concentrated to 350 ml, then applied to a column (5×41.5 cm) of DEAE-Sephadex A-25 (Pharmacia Co.). DEAE-Sephadex was used as the acetate form, produced by successive pretreatments with 0.5N sodium hydroxide, water, 1M acetate buffer (pH 5.0), and

water. After elution with water (1500 ml) and 0.2M acetate buffer (pH 5.0, 1880 ml), the column was eluted with 0.5M acetate buffer (pH 5.0, 1620 ml), 1M acetate buffer (pH 5.0, 900 ml), 2M acetate buffer (pH 5.0, 900 ml), and 0.2N sodium hydroxide (2600 ml). Fractions of 20 ml were collected and analyzed by the phenol-sulfuric acid method.[23] The eluates obtained from tubes 190 to 250 were combined, dialyzed against running water for two days, then concentrated and lyophilized. The yield (1.98 g) corresponds to 71% of the total amount of acidic polysaccharides obtained by the precipitation method with cetyltrimethyl ammonium bromide.[3] Though this fraction gave a single spot on electrophoresis, the following treatments were carried out for further purification.

The fraction (0.3 g) was dissolved in water and applied to a column (5×78 cm) of Sephadex G-25. The column was eluted with water and fractions of 20 ml were collected. The eluates obtained from tubes 28 to 47 were combined and concentrated to 25 ml. Four volumes of ethanol were poured into this solution, and the mixture was centrifuged. The same treatment with ethanol was repeated twice, then the precipitate was dissolved in water and lyophilized. Zizyphus-pectin A (0.22 g) was obtained as a white powder. It contains no nitrogen.

The eluates obtained from tubes 268 to 290 of the DEAE-Sephadex A-25 column chromatography were combined and treated similarly. A minor acidic polysaccharide (0.10 g) was obtained from this fraction.

Cellulose Acetate Membrane Electrophoresis——Cellulose acetate membrane electrophoresis was carried out with Separax in the manner described in previous reports[3,24] with a buffer of 0.08M pyridine–0.04M acetic acid (pH 5.4) at 420 V for 40 min. The sample was applied in a line at a distance of 7 cm from the cathode and gave a single spot at a distance of 3.5 cm toward the anode.

Glass-fiber Paper Electrophoresis——Glass-fiber paper electrophoresis was carried out with Whatman GF 83 glass-fiber paper in the manner described in a previous report[25] with the same pyridine-acetic acid buffer at 570 V for 90 min. The sample gave a single spot at a distance of 4.1 cm from the center toward the anode.

Gel Chromatography——The sample (2 mg) was dissolved in water and applied to a column (2.6×95 cm) of Toyopearl HW-65 (Tōyō Soda Co., fine). Elution was carried out by the ascending method with 0.1M potassium dihydrogen orthophosphate as an eluant. Fractions were collected at 5 ml and analyzed by the phenol-sulfuric acid method. The chromatogram is shown in Fig. 2. Standard dextrans having known molecular weights were run on the column to obtain the calibration curve shown in Fig. 3.

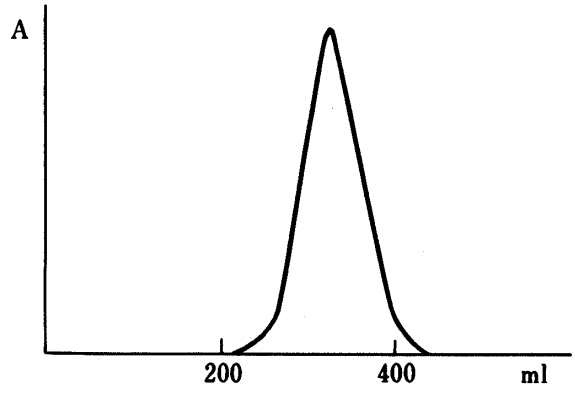

Fig. 2. Chromatogram of Zizyphus-pectin A on Toyopearl HW-65

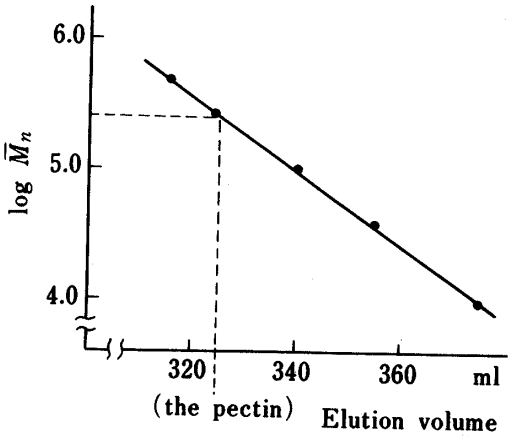

Fig. 3. Plot of Elution Volume against log M_n for Standard Dextrans on Toyopearl HW-65

Hydrolysis, Isolation and TLC of Component Sugars——These were carried out as described in a previous report.[26]

Determination of Component Sugars——Galacturonic acid and rhamnose were determined by colorimetric methods, and the other aldoses were analyzed by GLC, as described in a previous report.[11]

Determinaion of *O*-Acetyl Groups——The sample (2 mg) was hydrolyzed with 1N hydrochloric acid (0.05 ml) containing propionic acid as an internal standard in a sealed tube at 100°C for 2 h. The hydrolysate was directly subjected to GLC under the same conditions as described in a previous report.[27]

Determination of *O*-Methyl Groups in Methyl Esters——The sample (2 mg) was dissolved in 0.5N sodium hydroxide (0.1 ml) containing ethanol as an internal standard and stood at 20°C for 30 min. The hydrolysate was directly subjected to GLC. GLC was carried out under condition A, using a column (3 mm×2 m long spiral glass) packed with Porapak Q (80 to 100 mesh) at 120°C with a nitrogen flow of 20 ml per min; t_R (min), methanol 2.9; ethanol (internal standard) 7.0.

Carboxyl Reduction of the Pectin——The pectin (100 mg) was dissolved in 0.1N sodium hydroxide (3 ml) and kept at 12°C for 10 min. The solution was neutralized with 0.1N hydrochloric acid and diluted with water

up to 30 ml, then 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluenesulfonate (1 g) was added. The pH of the reaction mixture was maintained at 4.75 by titration with 0.1 N hydrochloric acid under stirring for 2 h, then 2 M sodium borohydride (10 ml) was added gradually to the reaction mixture during 4 h while the pH was maintained at 7.0 by titration with 4 N hydrochloric acid under stirring at room temperature. The solution was dialyzed against running water overnight, then the non-dialyzable fraction was concentrated to 30 ml. The product was reduced three times more under the same conditions. The final non-dialyzable fraction was applied to a column (5×74 cm) of Sephadex G-25. The column was eluted with water, and fractions of 20 ml were collected. The eluates obtained from tubes 27 to 31 were combined and lyophilized. Yield, 62 mg.

Methylation of the Pectin and its Carboxyl-reduced Product——Each sample (30 mg) was dissolved in dimethyl sulfoxide (3 ml). Sodium hydride (50 mg) was mixed with dimethyl sulfoxide (10 ml) in an ultrasonic bath for 30 min; the mixture was stirred at 70 °C for 1 h, then added to the sample solution. The reaction mixture was stirred at room temperature for 4 h, then methyl iodide (5 ml) was added and the whole was stirred overnight at room temperature. All procedures were carried out under nitrogen. After addition of water (30 ml), the reaction mixture was extracted six times with chloroform (30 ml each). The combined extract was washed five times with water (180 ml each), then dried over sodium sulfate, and the filtrate was concentrated to dryness. The residue was methylated four times more under the same conditions. The final residue was dissolved in chloroform–methanol mixture (2:1), then applied to a column (2×24 cm) of Sephadex LH-20. The column was eluted with the same solvent, and fractions of 3 ml were collected. The eluates obtained from tubes 9 to 14 were combined and concentrated to dryness. The final products (22 mg each from the both samples) were yellow powders. Their IR spectra showed no absorption of hydroxyl groups.

Analysis of the Methylated Products——Each product (10 mg) was hydrolyzed with dilute sulfuric acid in acetic acid, then reduced and acetylated in the manner described in a previous report[24] of this series. GLC–MS was carried out under the same conditions as in a previous report.[24] The relative retention times of the products with respect to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol in GLC and their main fragments in the mass spectra are listed in Table I.

TABLE I. Relative Retention Times on GLC and Main Fragments in MS of partially Methylated Alditol Acetates

	Relative retention times[a]	Main fragments (*m*/*z*)
1, 4-Ac-2, 3, 5-Me-L-Arabinitol	0.46	43, 45, 71, 87, 101,117, 129, 161
1, 4, 5-Ac-2, 3-Me-L-Arabinitol	1.06	43, 87, 101, 117, 129, 189
1, 3, 4, 5-Ac-2-Me-L-Arabinitol	1.74	43, 117, 261
1, 2, 5-Ac-3, 4-Me-L-Rhamnitol	0.88	43, 89, 129, 131, 189
1, 2, 4, 5-Ac-3-Me-L-Rhamnitol	1.60	43, 87, 101, 129, 143, 189, 203
1, 5-Ac-2, 3, 4, 6-Me-D-Galactitol	1.16	43, 45, 71, 87, 101, 117, 129, 145, 161, 205
1, 4, 5-Ac-2, 3, 6-Me-D-Galactitol	2.04	43, 45, 87, 99, 101, 113, 117, 233

a) Relative to 1, 5-di-*O*-acetyl-2, 3, 4, 6-tetra-*O*-methyl-D-glucitol.
Abbreviations: Ac=acetyl; Me=methyl (*e. g.*, 1, 4-Ac-2, 3, 5-Me-=1, 4-di-*O*-acetyl-2, 3, 5-tri-*O*-methyl-).

Acetylatoin and Chromium Trioxide Oxidation——The pectin (210 mg) was added in small portions to formamide (4 ml) at 60 °C during 1 h with stirring, then pyridine (3 ml) was added dropwise to the mixture at 50 °C during 30 min with stirring. Acetic anhydride (0.5 ml each) was added to the solution four times at 1 h intervals at 30 °C with stirring, then the reaction mixture was stirred overnight at room temperature. The solution was added dropwise to cold 2% hydrochloric acid (50 ml) and the mixture was stirred for 1 h. The resulting precipitate was separated by filtration, and suspended in water (30 ml) for 5 h. The final precipitate was separated and dried *in vacuo*. Yield, 261 mg. The acetylated pectin (17 mg) together with *myo*-inositol hexaacetate (0.3 mg) as an internal standard was dissolved in glacial acetic acid (2 ml) and held in an ultrasonic bath for 20 min, then stirred at room temperature for 3 h. Chromium trioxide (22 mg) was added to half of this solution. The reaction mixture was stirred at room temperature for 1 h, then poured into water (5 ml). The mixture was extracted three times with chloroform (5 ml each). The extracts were combined and washed sequentially with water (15 ml), saturated aqueous sodium hydrogen carbonate (15 ml), and water (15 ml each, three times). The chloroform solution was dried over sodium sulfate and the filtrate was evaporated to dryness. The residue was successively treated with 88% formic acid (2 ml) at 90 °C for 17 h and 2 M trifluoroacetic acid (2 ml) at 100 °C for 3 h. After evaporation and neutralization with Dowex 2 (OH^-), the hydrolysate was derivatized into alditol acetates and analyzed by GLC as described above.

Partial Acid Hydrolysis and Isolation of Oligosaccharides——The pectin (204 mg) was dissolved in 0.2 M trifluoroacetic acid (20 ml) and heated at 80 °C for 1 h. After removal of the acid by evaporation, the residue

was dissolved in water and applied to a column of Sephadex G-15 (5×82.5 cm). The column was eluted with water, and fractions of 20 ml were collected. The eluates obtained from tubes 28 to 37 were combined, concentrated, and lyophilized. This high molecular weight (MW) fraction (182 mg) contained all the galacturonic acid, rhamnose, and galactose residues, and 20% of the arabinose residues. The other eluates obtained from tubes 48 to 53 were combined, concentrated, and lyophilized. Yield, 20 mg. Arabinose was the sole component sugar in this fraction.

The secondary partial hydrolysis of the high MW fraction (900 mg) described above was performed with 0.2M trifluoroacetic acid (100 ml) at 100°C for 5 h. After removal of the acid as described above, the hydrolysate was applied to a column (5×82 cm) of Sephadex G-15. The column was eluted with water, and fractions of 20 ml were collected. The eluates obtained from the column were divided into three groups: the high MW Frac., tubes 28 to 34; the intermediate MW Frac., tubes 35 to 41; the low MW Frac., tubes 49 to 53. The yields were 405 mg for the high MW Frac., 250 mg for the intermediate MW Frac., and 110 mg for the low MW Frac.

The final partial hydrolysis of the intermediate MW fraction (115 mg) was performed with 2M trifluoroacetic acid (12 ml) at 100°C for 3 h. After removal of the acid as described above, the hydrolysate was applied to a column (1×11 cm) of DEAE-Sephadex A-25 (formate form). The column was eluted successively with water (25 ml), 0.1M formic acid (55 ml), 0.2M formic acid (35 ml), 0.3M formic acid (55 ml), 0.4M formic acid (35 ml), 0.5M formic acid (100 ml), 0.6M formic acid (35 ml), 0.8M formic acid (40 ml), 1M formic acid (70 ml), and 1.5M formic acid (60 ml), and fractions of 5 ml were collected. The eluates obtained from the column were divided into six groups: Frac. 1, tubes 1 to 3; Frac. 2, tubes 8 to 17; Frac. 3, tubes 25 to 34; Frac. 4, tubes 45 to 61; Frac. 5, tubes 79 to 88; Frac. 6, tubes 92 to 102. The yields were 21 mg for Frac. 1, 23 mg for Frac. 2, 12 mg for Frac. 3, 12 mg for Frac. 4, 10 mg for Frac. 5, and 8 mg for Frac. 6. Rhamnose was the sole component sugar in Frac. 1. Frac. 2 (10 mg) was dissolved in water and applied to a column (2.6×93.5 cm) of Sephadex G-15. The column was eluted with water and fractions of 5 ml were collected. The eluates obtained from the column were divided into two groups: Frac. a, tubes 48 to 55; Frac. b, tubes 61 to 66. The yields were 2.5 mg for Feac. a and 5 mg for Frac. b. Oligosaccharide I and galacturonic acid were obtained from Frac. a and b, respectively. Each of Fracs. 3, 4, 5, and 6 was separately applied to a column of Sephadex G-15 in the same manner. Oligosaccharides II, III, IV, and V were obtained in quantitative yields from Fracs. 3, 4, 5, and 6, respectively.

Cellulose TLC of Oligosaccharides II to V——TLC was carried out with Avicel SF by the ascending method at 26°C and developed 18 cm. The following solvent system was used; A, *n*-butanol : pyridine : water (1 : 1 : 1). The oligosaccharides were visualized with silver nitrate reagent.[28] Table II gives the R_{GalA} values on TLC and the values of specific rotation of the oligosaccharides.

TABLE II. Specific Rotations and R_{GalA} Values on TLC of the Oligosaccharides consisting of D-Galacturonic Acid

Oligosaccharides	Specific rotations in water (final values)		Cellulose TLC (R_{GalA}[a])
II	$[\alpha]_D^{22}$ +135.0°	(c=0.4)	0.76
III	$[\alpha]_D^{22}$ +181.3°	(c=0.3)	0.55
IV	$[\alpha]_D^{22}$ +192.3°	(c=0.3)	0.45
V	$[\alpha]_D^{22}$ +203.8°	(c=0.2)	0.34

a) Relative *Rf* values with respect to D-galacturonic acid.

Reducing Power Measurements of Oligosaccharides II to V——These were carried out by the method of Park and Johnson.[29] The values of reducing power relative to that of galacturonic acid were 56.9% in II, 35.9% in III, 26.5% in IV, and 21.4% in V.

Methylation Analysis of the Intermediate MW Fraction——This was carried out in the manner described above. The results are also listed in Table I.

Enzymic Degradation——The pectin (5 mg) was dissolved in 1 ml of 0.04M citrate-phosphate buffer (pH 3.0) containing 0.03% of α-L-arabinofuranosidase, and the solution was incubated at 40°C for 30 min. The rise of reducing activity was measured by the method of Park and Johnson.[29] Arabinose was the sole degradation product detected by TLC.

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