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

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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.* 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.* 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.

After removal of seeds, the fruits were sliced, homogenized and extracted with hot water. The extract was applied to a column of 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. 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 TLC of the hydrolysate and by GLC of its derivatives. These sugars were isolated by preparative 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-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 polysac-

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charide exist as methyl esters. The name "Zizyphus-pectin A" is proposed 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. Methylations of the original and the carboxyl-reduced polysaccharides were performed with methylsulfinyl carbanion and methyl iodide in dimethyl sulfoxide. The fully methylated products were hydrolyzed and the products were analyzed by GLC–MS after conversion into alditol acetates. 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.

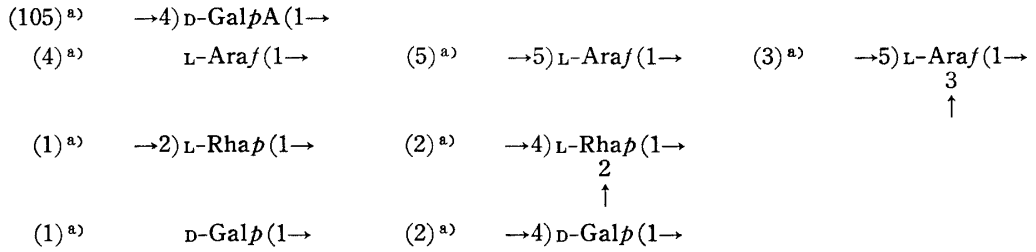

Chart 1. Component Sugar Residues in the Minimal Repeating Unit in the Structure of Zizyphus-pectin A
a) number of residues

The high positive value of the specific rotation suggests that D-galacturonic acid residues in the pectin are α-linked. 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. Angyal and James 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. In order to confirm the configuration of the arabinose linkages, the pectin was digested with the α-L-arabinofuranosidase from *Rhodotorula flava* 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 0.2 M 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 0.2 M 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 2 M 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.

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. I 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,* leaves and stems of *Medicago sativa,* roots of *Dianthus caryophyllus,* peels of *Citrus limon,* seed hulls of *Brassica campestris* subsp. *napus* var. *nippo-oleifera,* and suspension-cultured cells and leaves of *Nicotiana tabacum.* Thus pectins generally have similar backbones 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.

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.