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Author	友田, 正司(Tomoda, Masashi) 清水, 訓子(Shimizu, Noriko) 権田, 良子(Gonda, Ryoko)
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Plant Mucilages. XXXVI. Isolation and Characterization of a Mucilage, "Okra-mucilage R", from the Roots of *Abelmoschus esculentus**

Masashi TOMODA, Noriko SHIMIZU and Ryoko GONDA

友田正司，清水訓子，権田良子

In a previous paper of this series, the isolation and structural features of a representative mucilage, named Okra-mucilage F, from the immature fruits of *Abelmoschus esculentus* MOENCH (=*Hibiscus esculentus* L.; Okra) were reported. In contrast to several mucilages obtained by us from other plants in the Malvaceae family, Okra-mucilage F has a unique structure in having no branch at any of the D-galacturonic acid residues. The root of this plant also contains mucilages, but no structural study on the mucilages has been reported so far. We have now obtained a new representative mucilage from this material. The properties and main structural features of the mucilage are described in the present paper.

The mucilage was homogeneous as determined by ultracentrifugal analysis and gave a single spot on cellulose acetate membrane electrophoresis. Furthermore, it gave a single peak on gel chromatography with Sephacryl S-500. The mucilage showed a positive specific rotation ($[\alpha]_D^{23}$+10.0° in H_2O, c=0.1), and its solution in water gave the high intrinsic viscosity value of 33.4 at 30°C. Gel chromatography gave a value of approximately 1700000 for the molecular weight. The name "Okra-mucilage R" is proposed for this substance.

Galactose, rhamnose, galacturonic acid, and glucuronic acid were identified as the component sugars. The proton magnetic resonance (^{1}H-NMR) spectrum of the mucilage showed an acetyl signal at δ 2.16 (s) in addition to a methyl signal of rhamnose at δ 1.33 (d, J=6 Hz) and anomeric proton signals of the component sugars. Analysis of the acid hydrolysate of the mucilage by GLC showed the occurrence of acetic acid. Quantitative determination showed that the mucilage contained 26.4% galactose, 13.9% rhamnose, 15.2% galacturonic acid, 15.2% glucuronic acid, and 7.4% acetyl group, and that their molar ratio was 1.9 : 1.1 : 1.0 : 1.0 : 2.0. Determination of protein content was carried out by the method of Lowry *et al.*, and a value of 19.3% was obtained.

The carboxyl groups of hexuronic acid residues in the mucilage were reacted with a carbodiimide reagent, then reduced with sodium borohydride to give the corresponding neutral sugar units. Methylations of the original and the carboxyl-reduced mucilages were performed with methylsulfinyl carbanion and methyl iodide in dimethyl sulfoxide. The fully methylated products were hydrolyzed, reduced, and acetylated. The final

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products obtained were analyzed by GLC-MS. Methyl ethers of the hexuronic acids were removed from the hydrolysis products of the methylated original mucilage by treatment with an anion-exchange resin, and the residual products were identified as alditol acetates of 3,4-di-*O*-methyl-L-rhamnopyranose, 3-*O*-methyl-L-rhamnopyranose, 2,3,4,6-tetra-*O*-methyl-D-galactopyranose, and 2,3,6-tri-*O*-methyl-D-galactopyranose in a molar ratio of 1.0 : 2.1 : 2.2 : 3.9, while alditol acetates of 3,4-di-*O*-methyl-L-rhamnopyranose, 3-*O*-methyl-L-rhamnopyranose, 2,3,4,6-tetra-*O*-methyl-D-glucopyranose, 2,3,4,6-tetra-*O*-methyl-D-galactopyranose, 2,3,6-tri-*O*-methyl-D-galactopyranose, and 2,6-di-*O*-methyl-D-galacropyranose were identified in a molar ratio of 1.0 : 1.9 : 3.0 : 2.3 : 3.6 : 3.1 from the carboxyl-reduced product.

These results suggested that the minimal repeating unit of the polysaccharide moiety of Okra-mucilage R is composed of six kinds of component sugar units.

Partial hydrolysis of the mucilage was carried out with 1 N sulfuric acid for 2 h, then the reaction mixture was neutralized and applied to a column of Dowex 50 W (H^+). The eluate with water was applied to a column of DEAE-Sephadex A-25 (formate form). Five oligosaccharides (I to V) were obtained by stepwise elution with dilute formic acid, then purified by rechromatography. Based on the results of component sugar analysis, and by comparing their chromatographic properties, the ^{1}H-NMR spectra, and the values of specific rotation with those of authentic samples, I to V were identified.

All galactose residues were liberated from the mucilage under the conditions of the partial hydrolysis. In addition to the results of methylation analysis, this fact suggests the presence of galactosyl-(1→4)-galactosyl-(1→4)-galactose as the average length side chain or longer galactosyl side chains, having the same glycosidic linkages, linking to position 4 of two-thirds of the rhamnose residues in the backbone chain. The value of specific rotation of the galactose fraction was consistent with the D configuration.

The ^{1}H-NMR spectrum of the mucilage showed four anomeric proton signals at δ 4.48 (d, J=8 Hz), δ 4.71 (d, J=7 Hz), δ 5.00 (d, J=2 Hz), and δ 5.29 (d, J=3 Hz), and their integral ratio was 2 : 1 : 1 : 1. The signals at δ 4.71, 5.00, and 5.29 are due to β-D-glucuronic acid, α-L-rhamnose, and α-D-galacturonic acid residues, respectively. The signal at δ 4.48 suggests that the D-galactose residues in the mucilage are β-linked.

Based on the accumulated evidence described above, it can be concluded that the polysaccharide moiety of the mucilage has the following repeating unit (Chart 1).

The presence of side chains composed of (on average) three β-1→4 linked D-galactopyranosyl residues is a characteristic of the structure of Okra-mucilage R.

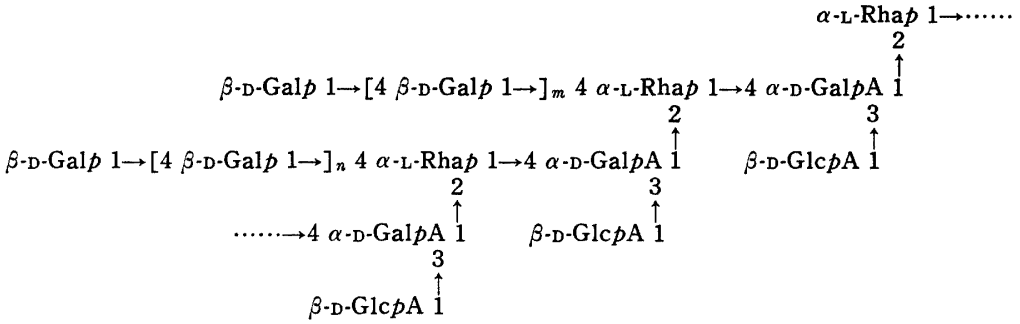

Chart 1. A Possible Structural Fragment of the Polysaccharide Moiety of Okra-mucilage R
m+n=4