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Author	友田, 正司(Tomoda, Masashi) 嶋田, 和代(Shimada, Kazuyo) 斉藤, 裕子(Saito, Yuko) 杉, 美智子(Sugi, Michiko)
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Plant Mucilages. XXVI. Isolation and Structural Features of a Mucilage, "Okra-mucilage F", from the Immature Fruits of *Abelmoschus esculentus**

Masashi Tomoda, Kazuyo Shimada, Yuko Saito, and Michiko Sugi

友田正司，嶋田和代，斉藤裕子，杉美智子

The immature fruits of *Abelmoschus esculentus* Moench (=*Hibiscus esculentus* L.; Okra) are widely used as a food. It is well known that the immature fruits contain relatively large amounts of mucilage. The mucilage has been used as a plasma expander and as an intravenous circulation agent.

Relatively many studies on the mucilage of this material have been published. However, the homogeneity and the mucosity of the mucilages obtained by the former investigators were uncertain, and differences in these factors presumably account for the disagreements in their conclusions.

We have now isolated a pure mucilage from the immature fruits of this plant. On the basis of the relative viscosity of the solution of the mucilage and its yield, we consider it is probable that the pure mucilage obtained by us is the major component accounting for the mucosity of the water extract from the material. Its properties and structural features are described in the present paper.

After removal of the seeds, the fresh immature fruits were sliced, homogenized and extracted with cold water. The crude mucilage was precipitated from the extract by addition of ethanol, then dissolved in dilute sodium sulfate solution. The solution was treated with cetyltrimethyl ammonium bromide, and the precipitate obtained was dissolved in sodium chloride solution. The resulting solution was poured into ethanol, then the precipitate was dissolved in water, reprecipitated with ethanol, and dialyzed against distilled water. A pure mucilage was obtained by lyophilization of the dialysate.

The mucilage was homogeneous as determined by ultracentrifugal analysis, and gave a single spot on cellulose acetate membrane electrophoresis in both a pyridine-acetic acid buffer and an alkaline borate buffer. Furthermore, it gave a single peak on gel chromatography with Sepharose 4B. It showed a positive specific rotation ($[\alpha]_D^{20}$ +51.8° in 1 N NaOH, c=0.1), and its solution in water gave the high intrinsic viscosity value of 30.1 at 30°. The relative viscosity of the solution of the pure mucilage was about 1.6 times that of the crude mucilage. Gel chromatography gave a value of approximately 1700000 for the molecular weight. The name "Okra-mucilage F" is proposed for this substance.

As component sugars of the mucilage, D-galactose, L-rhamnose, and D-galacturonic acid were identified by cellulose thin-layer chromatography of the hydrolysate. These sugars

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were isolated by preparative paper partition chromatography and shown to have the configurations given above. Quantitative determination showed that the mucilage contained 25.2% galactose, 21.8% rhamnose, and 27.3% galacturonic acid. The infrared spectrum has absorption bands at 1250 and 1730 cm^{-1}, suggesting the presence of ester linkages. When the acid hydrolysate was analyzed by gas-liquid chromatography, it gave a single peak, with a retention time equal to that of acetic acid. The acetyl content of the mucilage was determined to be 5.5%.

The mucilage contained 1.66% nitrogen. Determination of protein content was carried out and a value of 10.8% was obtained. No nitrogen-containing compound other than amino acids was detected in the hydrolysate. There is no significant difference in amino acid composition between Okra-mucilage F and Abelmoschus-mucilage M, except for the values of aspartic acid and glutamic acid.

The carboxyl groups of galacturonic acid residues in the mucilage were reacted with a carbodiimide reagent, then reduced with sodium borohydride to the corresponding neutral sugar units. Quantitative determination showed that the carboxyl-reduced mucilage was composed of 69.3% galactose and 30.7% rhamnose.

Methylations of the original and the carboxyl-reduced mucilages were performed with methylsulfinylmethyl sodium and methyl iodide in dimethyl sulfoxide. The fully methylated products were hydrolyzed with dilute sulfuric acid in acetic acid. The products were analyzed by gas-liquid chromatography-mass spectrometry after conversion into alditol acetates, and identified as 3,4-di-*O*-methyl-L-rhamnopyranose, 2,3,4,6-tetra-*O*-methyl-D-galactopyranose, 3-*O*-methyl-L-rhamnopyranose, and 2,3,6-tri-*O*-methyl-D-galactopyranose. They were obtained in a molar ratio of 1.00: 0.93: 1.05: 1.10 from the original mucilage, and in a molar ratio of 1.00: 1.01: 0.94: 3.16 from the carboxyl-reduced product. These results show that two-thirds of the trimethyl galactose units in the methylation product of the carboxyl-reduced mucilage are derived from galacturonic acid residues in the original material.

The mucilage was hydrolyzed with 1 N sulfuric acid for 2 hr, then neutralized and applied to a column of Dowex 50W (H^+). The eluate with water was applied to a column of DEAE-Sephadex A-25 (formate form). Only one oligosaccharide was obtained by stepwise elution with dilute formic acid, in addition to component sugars. 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, the oligosaccharide was identified as 2-*O*-α-(D-galactopyranosyluronic acid)-L-rhamnopyranose. The ratio of the yields of the disaccharide, galactose, rhamnose, galacturonic acid, and the residual water-insoluble fraction composed of rhamnose and galacturonic acid together with a small amount of amino acids was 2.2: 11.0: 1.0: 0.8: 19.0. On the other hand, a part of the solution of the partial hydrolysate was applied to a column of Sephadex G-25, and it was confirmed that the solution contained no polysaccharide fraction. Thus, no

galactose was found in any oligosaccharide or polysaccharide fraction after partial hydrolysis. On the other hand, about 90% of the rhamnose and galacturonic acid residues were present as components in the disaccharide and the water-insoluble polysaccharide fraction. These results suggest that branches in the polysaccharide moiety of the mucilage are composed of galactose residues.

The proton magnetic resonance (^{1}H-NMR) spectrum of the carboxyl-reduced mucilage showed three anomeric proton signals at δ 4.76 (d, J=8 Hz), δ 5.06 (d, J=2 Hz), and δ 5.32 (d, J=2 Hz), an acetyl signal at δ 2.22 (s), and a methyl signal at δ 1.37 (d, J= 6 Hz). The ratio of their integrals was 1.0: 1.0: 1.0: 1.5: 3.0. Half of the D-galactose units must be β-linked, because of the high value observed for the coupling constant of the signal at δ 4.76 in the ^{1}H-NMR spectrum. This result also suggests that the remaining D-galactose units are α-linked (the signal at δ 5.32 in the ^{1}H-NMR spectrum), and that L-rhamnose units are α-linked (the signal at δ 5.06 in the ^{1}H-NMR spectrum) in the substance. Based on the results of the partial hydrolysis, it is evident that D-galacturonic acid residues are α-linked in the original mucilage, so D-galactose residues in the mucilage must be β-linked.

The mucilage was subjected to periodate oxidation followed by reduction with sodium borohydride. The maximal periodate consumption was 0.74 mol per mol of anhydrosugar unit. The reduction product was isolated by gel chromatography on Sephadex G-15. It contained 16.7% rhamnose, 9.6% galacturonic acid, and 5.3% *O*-acetyl groups; their molar ratio was thus 2.1: 1.0: 2.2. In addition, a small proportion (3.8%) of protein still remained, but no galactose was found in it. These results indicate that about two-thirds of L-rhamnose and about one-third of D-galacturonic acid in the mucilage survived after periodate oxidation. It can be deduced that the *O*-acetyl groups are attached in such a manner that they block periodate cleavage of the surviving sugar units at positions 3 or 4 of about one-third of straight chain rhamnose residues, at position 3 of about one-third of branched rhamnose residues, and at positions 2 or 3 of about one-third of galacturonic acid residues.

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

The presence of a main chain having the repeating structure (1→4)-*O*-α-(D-galacto-

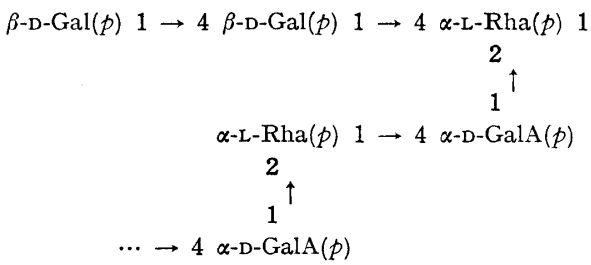

Chart 1. A Possible Structural Fragment of the Polysaccharide Moiety of Okra-mucilage F

pyranosyluronic acid)-(1→2)-*O*-α-L-rhamnopyranose is common in the mucilages from the roots of *Althaea officinalis*, *Abelmoschus manihot*, and *Abelmoschus glutinotextilis*, from the fruit of *Abelmoschus esculentus*, and from the inner bark of *Hydrangea paniculata*. In the structures of Althaea-mucilage O, Abelmoschus-mucilage M, and paniculatan, all the D-galacturonic acid residues possess β-D-glucuronic acid branches at position 3. In Abelmoschus-mucilage G, three-quarters of the D-galacturonic acid residues possess β-D-glucuronic acid branches at position 3. In contrast to these mucilages, Okra-mucilage F has no branch at the D-galacturonic acid residues. It has branches composed of 4-*O*-β-D-galactopyranosyl D-galactopyranose at position 4 of half the L-rhamnose units in the main chain. Among the mucilages described above, Althaea-mucilage O possesses similar galactosyl galactose branches at position 4 of about one-third of the L-rhamnose units.