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Plant Mucilages. XXV. Isolation and Structural Features of a Mucilage, "Abelmoschus-mucilage G", from the Roots of Abelmoschus glutinotextilis*

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In the previous papers of this series, the isolation and structural features of a representative mucilage, named Abelmoschus-mucilage M, from the roots of *Abelmoschus manihot* have been reported from this laboratory. In the present study we obtained a new representative mucilage from the roots of *Abelmoschus glutinotextilis* Kagawa. Its properties and main structural features are described in the present paper. The extract with water from the root of this plant contains many mucilages. It has been used as a size similar to that obtained from the root of *Abelmoschus manihot*, and its fruit is used as a food.

The fresh roots were crushed 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 acetone, then the precipitate was dissolved in water, reprecipitated with acetone, 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 Sephadex G-200. It showed a positive specific rotation ($[\alpha]_0^{20} + 53.3^{\circ}$ in H₂O, c=0.1), and its solution in water gave the high intrinsic viscosity value of 52.8 at 30°. The relative viscosity of the solution of the pure mucilage was about 2.2 times that of the crude mucilage. In view of this result and the yield, it is conceivable that the pure mucilage is the representative mucous substance in the water extract from the roots. Gel chromatography gave a value of 67900 for the molecular weight. The name "Abelmoschus-mucilage G" is proposed for this substance.

As component sugars of the mucilage, L-rhamnose, p-galacturonic acid, and p-glucuronic acid were identified by cellulose thin-layer chromatography of the hydrolysate. These sugars were isolated by preparative paper partition chromatography and proved to have the configurations given above.

The carboxyl groups of hexuronic acid residues in the mucilage were reacted with a

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carbodiimide reagent, then reduced with sodium borohydride to the corresponding neutral sugar units. Quantitative determinations of the neutral component sugars of the original and the carboxyl-reduced mucilages were carried out by gas-liquid chromatography of alditol acetates derived from the hydrolysates, and hexuronic acids in the original mucilage were estimated by a colorimetric method. The results showed that the mucilage contained 24.5% rhamnose and 51.1% hexuronic acids, and that the molar ratio of rhamnose: galactose: glucose was 10.0: 9.8: 7.6 in the carboxyl-reduced mucilage.

The mucilage contained 2.88% nitrogen. The determination of protein content was carried out and a value of 19.4% 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 Abelmoschus-mucilage G and Abelmoschus-mucilage M except for the values of aspartic acid, methionine and tyrosine.

The methylation of the carboxyl-reduced mucilage was performed with methylsulfinyl-methyl sodium and methyl iodide in dimethylsulfoxide. The fully methylated product was hydrolyzed successively with formic acid and dilute sulfuric 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-glucopyranose, 2,3,6-tri-O-methyl-D-galactopyranose, and 2,6-di-O-methyl-D-galactopyranose. They were obtained in a molar ratio of 4.0:3.0:1.0:3.0.

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). Four oligosaccharides (I to IV) were obtained

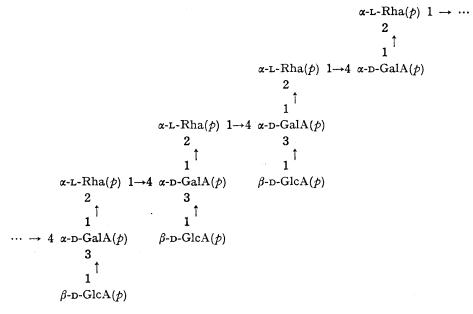


Chart 1. A Possible Structural Fragment of the Polysaccharide Moiety of Abelmoschus-mucilage G

by stepwise elution with dilute formic acid and were purified by rechromatography. Based on the results of component sugar analysis, and by comparing their chromatographic and electrophoretic properties and the values of specific rotation with those of authentic samples, I to IV were identified.

Based on the accumulated evidence described above, it can be concluded that the poly-saccharide moiety of the mucilage has a main chain composed of $(1\rightarrow 4)-O-\alpha-(D-galacto-pyranosyluronic acid)-(1\rightarrow 2)-O-\alpha-L-rhamnopyranosyl repeating units. Three-quarters of the D-galacturonic acid residues in the main chain possess <math>\beta$ -D-glucuronic acid residues at position 3, but a quarter of the D-galacturonic acid residues has no branch. A possible structure of the polysaccharide moiety of Abelmoschus-mucilage G is shown in Chart 1.

The component unit having the repeating structure $(1\rightarrow 4)$ - $[O-\beta-(D-glucopyranosyluronic acid)-(1\rightarrow 3)]-O-\alpha-(D-galactopyranosyluronic acid)-<math>(1\rightarrow 2)$ - $O-\alpha$ -L-rhamnopyranose is common in the mucilages from the roots of Abelmoschus glutinotextilis, Abelmoschus manihot, and Althaea officinalis, and from the inner bark of Hydrangea paniculata. Abelmoschus-mucilage M appears to possess the simplest repeating structure in its polysaccharide moiety. On the other hand, the partial lack of branches at position 3 of the D-galacturonic acid residues in the main chain is characteristic of Abelmoschus-mucilage G.