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Plant Mucilages. XVIII. Isolation and Characterization of a Mucilage, "Abelmoschus-mucilage M", from the Roots of *Abelmoschus manihot* *

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The root of *Abelmoschus manihot* MEDICUS (=*Hibiscus manihot* L.) contains fairly large amounts of mucilage. It has been used as a crude drug for the purpose of demulcent and cough medicine. Further, the mucous solution extracted from it with water has been used as an important size for the traditional paper manufacture in Japan.

Relatively many studies on the root mucilage of this plant have been published until present time. Nevertheless, the homogeneity of the mucilages obtained by the former investigators was uncertain, and no further structural study on the whole mucilage has been reported untill now. We have now isolated a pure mucilage having glucuronic acid in addition to galacturonic acid and rhamnose as its component sugars. From the viscosity and the yield, it is probable that the mucilage is the representative substance in the mucosity of water extract from the material. The properties and the main structural features of it are described in the present paper.

The fresh roots were crushed and extracted with cold water. The crude mucilage was precipitated from the extract by addition of ethanol. The solution of the precipitate was applied to a column of DEAE-Sephadex A-25 (carbonate form). Negligibly small amounts of the substances adsorbed were eluted with water and 0.1 M ammonium carbonate solution, and the main mucilage was obtained from the eluate with 0.5 M ammonium carbonate solution. A minor mucilage was obtained from the eluate with 1 M ammonium carbonate solution. The same component sugars as those of the main mucilage were found in it, but no further structural study on this substance was carried out.

The main mucilage was homogeneous by the ultracentrifugal analysis, and gave a single spot on cellulose acetate membrane electrophoresis. Its solution in water gave the high intrinsic viscosity value of 33.0 at 31°, and it showed a positive specific rotation ($[\alpha]_D^{22}$ + 51.7° in 0.1% NH_4OH, $c=0.1$). The relative viscosity of the solution of the main mucilage was about 3.7 times as high as the value of the crude mucilage. The water solution of the minor mucilage gave the intrinsic viscosity value of 23.0 at the same condition.

As the component sugars of the main mucilage, L-rhamnose, D-galacturonic acid, and D-glucuronic acid were identified by means of cellulose thin-layer chromatography (TLC) of the hydrolyzate. These sugars were isolated by preparative paper partition chromatography (PPC) and proved to have the configurations given above. Quantitative determination of the component sugars showed that the molar ratio of rhamnose : galacturonic acid :

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glucuronic acid is 1.2 : 1.0 : 1.0, and that the total content of carbohydrates is 82.0%.

The mucilage contained 2.69% of nitrogen. Any nitrogen-containing compound, other than amino acids, was not detected in the hydrolyzate. The total value of the amino acid composition of it after hydrolysis with 6 N hydrochloric acid was less than one third of the value expected from nitrogen content. The cause for such a discrepancy may be attributed to unavoidable decomposition of amino acids arising from the presence of a large quantity of carbohydrates.

The measurement of osmotic pressure gave the value of 25300 as the molecular weight of the ammonium salt of the mucilage. The name "Abelmoschus-mucilage M" is proposed for it.

The carboxyl groups of hexuronic acid residues in the mucilage were reacted with a carbodiimide reagent, then reduced with sodium borohydride to the corresponding neutral sugar units. The methylation of the carboxyl-reduced mucilage was performed with methylsulfinylmethyl sodium and methyl iodide in dimethyl sulfoxide. The fully methylated product was successively hydrolyzed with formic acid and dilute sulfuric acid. The products derived from component sugars were analyzed by gas-liquid chromatography-mass spectrometry (GLC-MS) after conversion to alditol acetates, and identified as 3, 4-di-*O*-methyl-L-rhamnopyranose, 2,3,4,6-tetra-*O*-methyl-D-glucopyranose, and 2,6-di-*O*-methyl-D-galactopyranose. They were obtained in the molar ratio of 1.1 : 1.0 : 1.0.

Owing to these results, it can be concluded that Abelmoschus-mucilage M is composed of 82% of acidic polysaccharide and approximately 17% of protein (from N, 2.69%). The polysaccharide chain in it must be composed of 1→2 linked L-rhamnopyranose units and 1→4 linked D-galactopyranosyluronic acid units having D-glucopyranosyluronic acid residues at position 3.

The presence of the component unit composed of 1→2 linked L-rhamnose and 1→4 linked D-galacturonic acid having D-glucuronic acid moiety has been reported in the cases of the gum from *Sterculia urens*, and of the mucilages from inner barks of *Hydrangea paniculata*, and roots of *Althaea officinalis*. In these example, D-galactose, or D-galactose and 4-*O*-methyl-D-glucuronic acid were also found as the other component sugars. Thus the manner of the carbohydrate linkage in Abelmoschus-mucilage M is not so complicated as these three polysaccharides, but it belongs to one of the complexes with carbohydrate and peptide.