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Plant Mucilages. XLI. A Mucilage from *Hibiscus moscheutos* Leaves*

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Recently, Tomoda *et al.* have obtained a representative mucilage, named "Hibiscus-mucilage Mo," from the roots of *Hibiscus moscheutos* L. (Malvaceae), and its structural features have been reported. In addition, the leaves of this plant contain relatively large amounts of mucilages.

In this paper, we report the isolation, structural features and hypoglycemic activity of a new representative mucilage from the leaves of *Hibiscus moscheutos* L.

The aqueous solution of crude mucilage was applied to a column of DEAE-Sephadex A-25 (carbonate form). After elution with water and 0.2 M ammonium carbonate, the eluate with 0.5 M ammonium carbonate was dialyzed and purified by successive gel chromatography with Cellulofine GCL-2000 m and Sephadex G-25.

The mucilage gave a single spot on zone electrophoresis with glass-fibre paper,and in addition, it gave a clear single band on PAGE. Both the periodate-Schiff reagent and the Coomassie blue reagent visualized a band in the same position. Further, it gave a single peak on gel chromatography with Sephacryl S-400. The mucilage showed a positive specific rotation ($[\alpha]_D^{24}+54.5°$) and its aqueous solution gave the high intrinsic viscosity value of 26.1 at 30°. Gel chromatography using standard dextrans gave a value of *ca.* 1800000 for its M_r. The name "Hibiscus-mucilage ML" is proposed for this substance.

Quantitative analyses showed that the mucilage was composed of a polysaccharide (90.6%) and a peptide moiety (8.6%). The polysaccharide moiety was composed of L-rhamnose (26.7%), D-galactose (19.7%), D-glucose (1.7%), D-galacturonic acid (21.5%), D-glucuronic acid (19.7%) and *O*-acetyl groups (1.3%). Their molar ratio was 18 : 12 : 1 : 12 : 11 : 3.

Both the original mucilage and the carboxyl-reduced derivative were methylated with methylsulphinyl carbanion and methyl iodide in dimethyl sulphoxide. The methylated products were hydrolysed, then converted into the partially methylated alditol acetates. GC/MS showed the presence of 3,4-di-*O*-methyl rhamnose, 3-*O*-methyl rhamnose, 2,3,4,6-tetra-*O*-methyl galactose, 2,3,6-tri-*O*-methyl galactose and 2,3,6-tri-*O*-methyl glucose as the products from the original mucilage in a molar ratio of 16 : 2 : 2 : 10 : 1. The carboxyl-reduced derivative gave 3,4-di-*O*-methyl rhamnose, 3-*O*-methyl rhamnose, 2,3,4,6-tetra-

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O-methyl glucose, 2,3,4,6-tetra-*O*-methyl galactose, 2,3,6-tri-*O*-methyl galactose, 2,3,6-tri-*O*-methyl glucose and 2,6-di-*O*-methyl galactose in a molar ratio of 16 : 2 : 11 : 2 : 11 : 1 : 11.

The mucilage was partially hydrolysed with dilute sulphuric acid, then neutralized and treated with Dowex 50 W (H^+). The eluate with water was applied to a column of DEAE-Sephadex A-25 (formate form). In addition to a part of component monosaccharides, four oligosaccharides (I to IV) were obtained by stepwise elution with dilute formic acid. Based on the results of component sugar analysis and a comparison of their chromatographic properties, their ^{1}H-NMR spectra and their values of specific rotation with those of authentic samples, I to IV were identified.

^{1}H-NMR spectrum of the mucilage showed four anomeric hydrogen signals at δ 4.69 (*d*, J=7 Hz), 4.71 (*d*, J=7 Hz), 5.00 (*d*, J=2 Hz) and 5.15 (*bs*). Among them, it was evident that those at δ 4.69 and 4.71 were attributable to anomeric protons of β-D-glucuronic acid, β-D-galactose and β-D-glucose, those at δ 5.00 and 5.15 to anomeric protons of α-L-rhamnose and α-D-galacturonic acid.

Based on the accumulated evidence described above, it may be concluded that the polysaccharide moiety of the mucilage contains the units shown in Fig. 1.

When administered *i.p.* (10, 30, 100 mg/kg), Hibiscus-mucilage ML reduced the blood glucose concentration dose-dependently (after 7 hr : 91, 74, 49%, after 24 hr : 100, 92, 68% of the control). The majority of the mucilages from plants in the Malvaceae family exhibited significant hypoglycemic activity on *i.p.* injection to normal mice. Thus Hibiscus-mucilage ML also showed remarkable hypoglycemic activity, and its effect was typically dose-dependent.

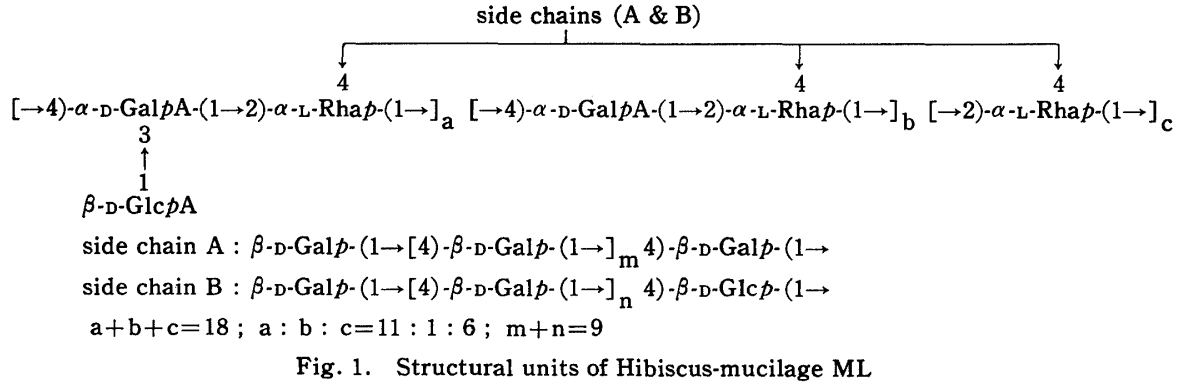

Fig. 1. Structural units of Hibiscus-mucilage ML