Title	Plant mucilages. XXX. isolation and characterization of a mucilage, "dioscorea-mucilage B," from the rhizophors of dioscorea batatas
Sub Title	
Author	友田, 正司(Tomoda, Masashi) 石川, 和代(Ishikawa, Kazuyo) 横井, 真恵美(Yokoi, Maemi)
Publisher	共立薬科大学
Publication year	1981
Jtitle	共立薬科大学研究年報 (The annual report of the Kyoritsu College of Pharmacy). No.26 (1981.) ,p.74- 76
JaLC DOI	
Abstract	
Notes	抄録
Genre	Technical Report
URL	https://koara.lib.keio.ac.jp/xoonips/modules/xoonips/detail.php?koara_id=AN00062898-00000026-0074

Plant Mucilages. XXX. Isolation and Characterization of a Mucilage, "Dioscorea-mucilage B," from the Rhizophors of *Dioscorea batatas**

Masashi Tomoda, Kazuyo Ishikawa, and Maemi Yokoi

友田正司，石川和代，横井真恵美

The tuberous rhizophors of *Dioscorea batatas* Decaisne are used as a crude drug for the purpose of antidiarrheic, antidipticum, tonic, and cough medicine, and are also widely used as a food. It is well known that the rhizophors contain a relatively large amount of mucilage. We have now isolated a pure mucilage from the rhizophors of this plant. From 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. This paper is concerned with the constitution of the mucilage, particularly the structural features of a polysaccharide moiety in it.

The fresh rhizophors were homogenized and extracted with cold water. The crude mucilage was precipitated from the extract by addition of ethanol, then dissolved again in water. The solution was treated with sodium lauryl sulfate and sodium chloride, and after centrifugation, the supernatant obtained was poured into ethanol. The precipitate obtained was dissolved in water and reprecipitated twice with ethanol. A pure mucilage was obtained by lyophilization.

The mucilage was homogeneous as determined by ultracentrifugal analysis, and gave a single band on polyacrylamide gel electrophoresis. Furthermore, it gave a single peak on gel chromatography with Sepharose 4B. It showed a negative specific rotation ($[\alpha]_D^{20}$ $-47.3°$ in H_2O, $c=0.05$), and its solution in water gave the intrinsic viscosity value of 21.0 at 30°. The relative viscosity of the solution of the pure mucilage was about 2.5 times that of the crude mucilage. Gel chromatography gave a value of approximately 2000000 for the molecular weight. The name "Dioscorea-mucilage B" is proposed for this substance.

The mucilage contained 64.0% protein, 26.2% mannan, 2.7% acetyl group, and 1.2% phosphorus. Its amino acid composition after hydrolysis with 6 N hydrochloric acid showed that there was no significant difference in amino acid composition between Dioscorea-mucilage B and the mucilage from *Dioscorea batatas* forma *Tsukune*, except for the values of cysteine, methionine, and tryptophan. On the other hand, a pronounced difference in composition was found as compared with the mucilage from *Dioscorea batatas* forma *Icho*.

The carbohydrate moiety in the mucilage was isolated by treatment with Pronase followed by gel chromatography with Sephadex G-50. The polysaccharide fraction obta-

* 本報告は *Chem. Pharm. Bull.*, **29**, 3256—3261 (1981) に発表。

ined was homogeneous as determined by ultracentrifugal analysis, and gave a single spot on glass-fiber paper electrophoresis. Furthermore, it gave a single peak on gel chromatography with Sepharose 4B. Gel chromatography gave a value of approximately 99000 for its molecular weight. It showed a negative specific rotation ($[\alpha]_D^{19}$ $-29.3°$ in 0.1% NH_4OH, $c=0.2$).

Its infrared (IR) spectrum has absorption bands at 1250 and 1735 cm^{-1}, suggesting the presence of ester linkages, in addition to the band of 890 cm^{-1} due to the presence of β-glycosidic linkages. When its acid hydrolysate was analyzed by gas-liquid chromatography (GLC), it gave a single peak, with a retention time equal to that of acetic acid.

Quantitative determination of the components showed that the polysaccharide moiety contained 84.8% mannose, 6.8% acetyl group, and 1.4% of a peptide composed of serine, threonine, glycine, alanine, valine, aspartic acid, glutamic acid, and lysine.

Methylation of the polysaccharide was performed with methylsulfinyl carbanion and methyl iodide in dimethyl sulfoxide. The fully methylated product was hydrolyzed with dilute sulfuric acid in acetic acid. The products were analyzed by gas-liquid chromatography-mass spectrometry (GLC–MS) after conversion into alditol acetates, and identified as 2,3,4,6-tetra-*O*-methyl-D-mannopyranose, 2,3,6-tri-*O*-methyl-D-mannopyranose, and 2,6-di-*O*-methyl-D-mannopyranose. They were obtained in a molar ratio of 1.0: 1.9: 1.0.

The polysaccharide was peracetylated with acetic anhydride and pyridine in formamide, then partially degraded with sulfuric acid in acetic anhydride. After deacetylation, the products were analyzed by thin-layer chromatography (TLC) and by GLC of trimethylsilylated derivatives. Comparison with authentic samples showed the presence of D-mannose, *O*-β-D-mannopyranosyl-(1→4)-D-mannopyranose, and *O*-β-D-mannopyranosyl-(1→4)-*O*-β-D-mannopyranosyl-(1→4)-D-mannopyranose.

The polysaccharide was treated with a β-D-mannanase obtained from Driselase in order to improve the low solubility in organic solvents. Then the product was exhaustively treated with methyl vinyl ether in the presence of *p*-toluenesulfonic acid in dimethyl sulfoxide. After conversion of the free hydroxyl groups into 1-methoxyethyl ethers, the derivative was deacetylated, then methylated with methyl iodide and silver oxide in *N*,*N*-dimethylformamide. The resulting product was hydrolyzed and analyzed by GLC and GLC–MS after conversion into alditol acetates as described above. Two hexose methyl ethers were detected and identified as 6-*O*-methyl-D-mannose and 2,3,6-tri-*O*-methyl-D-mannose in a molar ratio of 1.0: 1.2. The results indicate that some residues of 6-*O*-acetyl-D-mannose and 2,3,6-tri-*O*-acetyl-D-mannose are present in the polysaccharide.

Based on these results, it can be concluded that the polysaccharide moiety is mainly composed of partially acetylated β-1→4 linked D-mannopyranose units having about one fourth degree of branching at the C-3 positions.

Misaki *et al.* isolated a partially acetylated mannan from the mucilage of the tubers of *Dioscorea batatas* forma *Tsukune*. They suggested that the acetyl groups were located

at positions 2 and/or 3, but definitive elucidation of the location has not been achieved. The polysaccharide moiety obtained by us possesses higher values of both molecular weight and degree of branching than the mannan moiety obtained by them, though the manner of branching is similar in both. The location of acetyl groups in Dioscorea-mucilage B is also different from that in the mucilage obtained from *Dioscorea batatas* forma *Tsukune*.

The mode of polysaccharide-protein linkage or the possibility of polysaccharide-protein complex formation by some type of molecular association in Dioscorea-mucilage B remains to be investigated. Further work is in progress.