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Plant Mucilages. I. Isolation and Property of a Mucous Polysaccharide, "Plantasan," from *Plantago major* L. var. *asiatica* Seeds

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An acidic polysaccharide has been isolated from the seed of *Plantago major* L. var. *asiatica* Decaisne. The polysaccharide, named plantasan, was homogenous on gel chromatography and glass-fiber paper electrophoresis, and it was the representative mucilage of the seed. The component carbohydrates of it were D-xylose, L-arabinose, D-galacturonic acid, L-rhamnose and D-galactose, and the molar ratio of them was 15:3:4:2:0.4.

The seed mucilages of *Plantago* genus were studied widely,[2] but no report on the chemical property of the mucilage of *Plantago major* L. var. *asiatica* Decaisne seeds has been published until present time. The seeds of this species have been used as a crude drug for the purpose of antiphlogistic, expectorant, diuretic, cathartic and binding medicine. The presences of plantenolic acid, succinic acid, adenine, choline, aucubin, and oils composed of glycerides of palmitic acid, stearic acid, arachidic acid, oleic acid and linolenic acid were reported by former investigators.[3] We have now isolated a mucous polysaccharide from this material, and its properties are described in the present paper.

The seeds were extracted with water, and after treatment with dilute alkali, a supernatant was obtained by centrifugation. An acidic polysaccharide was prepared from the solution by repeated precipitation with ethanol containing hydrochloric acid. For the purpose of the isolation of the main polysaccharide, the applications of DEAE-cellulose column chromatography or of a precipitation method with cetylpyridinium chloride have also been attempted, but it was found that the precipitation method with acidic ethanol is the best in respect of both the yield of pure product and the simplicity of experimental procedure. The polysaccharide was homogeneous on molecular-sieve chromatography with Sephadex G-200 and gave one spot on glass-fiber paper electrophoresis in alkaline borate buffer. The name "plantasan" is proposed for the polysaccharide. It showed a negative specific rotation ($[\alpha]_D^{24}$ $-55.8°$ in 1N NaOH, $c=1$).

The supernatant and washings with acidic ethanol were combined and dialyzed, then applied to a DEAE-cellulose column. Although several fractions were obtained with water and gradient elution of alkaline solvent, the main fraction was heterogeneous on gel-permeation chromatography with Sephadex G-100. The outline of the fractionation is shown in Chart 1.

It was shown that the component sugars of plantasan are D-xylose, L-arabinose, D-galacturonic acid, L-rhamnose and D-galactose by means of cellulose thin-layer chromatography of the hydrolysate. The same five component sugars were also found in the polysaccharide

1) Location: *Shibakoen, Minato-ku, Tokyo.*

2) *a*) J. Mullan and E.G.V. Percival, *J. Chem. Soc.*, **1940**, 1501; *b*) E. Anderson, L.A. Gillette and M.G. Seeley, *J. Biol. Chem.*, **140**, 569 (1941); *c*) W.A.G. Nelson and E.G.V. Percival, *J. Chem. Soc.*, **1942**, 58; *d*) R.A. Laidlaw and E.G.V. Percival, *J. Chem. Soc.*, **1949**, 1600; *e*) E.G.V. Percival and I.C. Willox, *J. Chem. Soc.*, **1949**, 1608; *f*) F. Hostettler and H. Deuel, *Helv. Chim. Acta*, **34**, 2440 (1951); *g*) E.L. Hirst, E.G.V. Percival and C.B. Wylam, *J. Chem. Soc.*, **1954**, 189; *h*) A.J. Erskine and J.K.N. Jones, *Can. J. Chem.*, **34**, 821 (1956).

3) *a*) A. Ogata and R. Nishioji, *Yakugaku Zasshi*, **44**, 1040 (1924); *b*) T. Kashimoto, *Nihon Kagaku Zasshi*, **76**, 664 (1955).

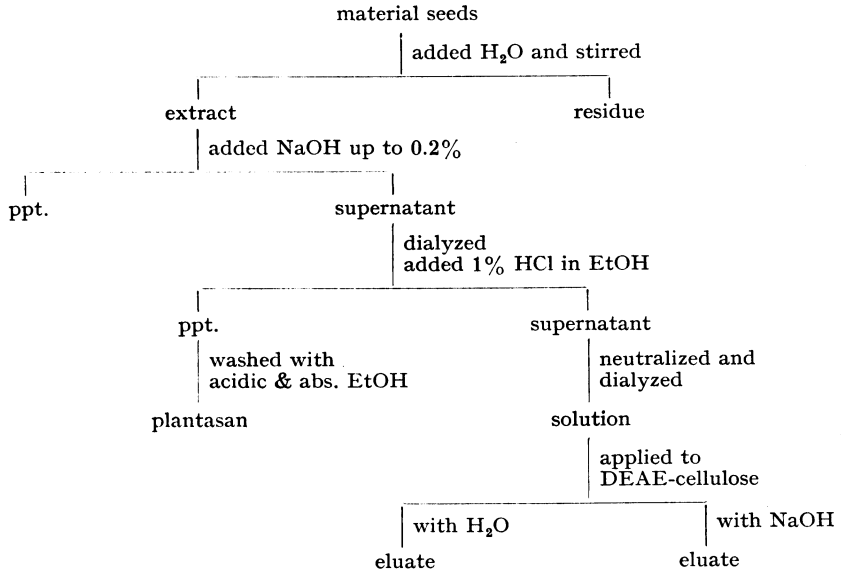

Chart 1. Isolation and Fractionation of Polysaccharides

fractions obtained from the DEAE-cellulose column, but because of their low yields and heterogeneity, no further investigation on the other polysaccharides than plantasan was carried out.

Molecular-sieve chromatography of standard dextran fractions of known molecular weights on Sephadex G-200 has given the calibration curve shown in Fig. 1. The number-average molecular weight, $\bar{M}n$, of plantasan (sodium salt) thus estimated was *ca.* 300000, and the value was also supported by the measurement of osmotic pressure.

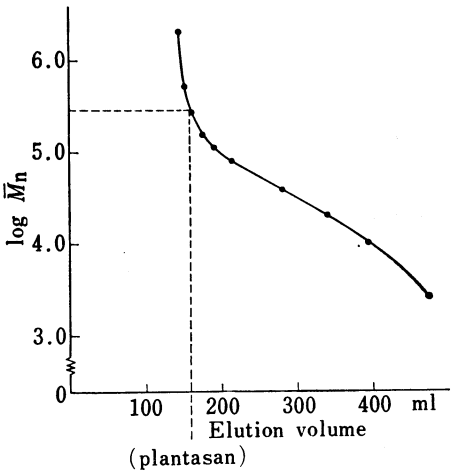

Fig. 1. Plot of Elution Volume against log $\bar{M}n$ for Dextran Fractions on Sephadex G-200 with 0.1 M Ammonium Formate

Quantitative determinations of the sugar components of plantasan showed that the molar ratio of them was as follows; D-xylose: L-arabinose: D-galacturonic acid: L-rhamnose: D-galactose was about 15:3:4:2:0.4. The neutral water solution of plantasan (sodium salt) gave the intrinsic viscosity value of 32.0 at 20°. And the relative viscosity of the solution of the polysaccharide is six times as high as the value of the crude water extract from the seed in the same condition. From this result and the yield of the polysaccharide, it is conceivable that plantasan is the representative substance in the mucosity of water extract from the seed.

The kind of component sugars of plantasan and the fact that D-xylose is the major constituent are similar to the properties of polysaccharides obtained from the seeds of the other species of *Plantago* genus, but the content of D-galacturonic acid is the highest among others. The detailed structure of the mucous polysaccharide will be discussed in following papers.

Experimental

Solutions were evaporated at 40° or below with rotary evaporators under reduced pressure. Specific rotation was measured using JASCO model DIP-SL automatic polarimeter.

Isolation of Plantasan——The seeds (10 g) of *Plantago major* L. var. *asiatica* were extracted with water (200 ml) by stirring for 24 hr at room temperature. After passing through a sieve of 50 meshes, a turbid, mucous filtrate was obtained. The filtrate was added with 1 N sodium hydroxide up to 0.2% and centrifuged at 10000 rpm (17000×g) for 40 min at 5°. The supernatant was dialyzed against running water for 4 days, then added with 300 ml of ethanol containing 1% hydrochloric acid and centrifuged as described above. The precipitate was dispersed into water (30 ml) and again added with the same volume of the acidic ethanol, then centrifuged similarly. After three times repetition of this procedure, the precipitate was repeatedly washed with absolute ethanol, and dried *in vacuo* in a desiccator. Then, it was dispersed into small amount of water and lyophilized. Yield, 6.8%. The polysaccharide was obtained as grayish white powder.

Chromatography of DEAE-cellulose——The supernatant and washings obtained after separation of plantasan were combined and concentrated, then neutralized with 0.4 N sodium hydroxide. After dialysis for one day, the solution was concentrated to 20 ml and applied to a column (2.6×80 cm) of DEAE-cellulose (Brown Co.). DEAE-cellulose was previously treated with 0.5 N sodium hydroxide and the excess of alkali was removed by repeated washing with water. The column was eluted with water, followed by gradient elution with sodium hydroxide. Fractions of 20 ml were collected and analyzed by phenol–sulfuric acid method.[4] The result was shown in Fig. 2. The yield of eluate with water was 0.3% and that of eluate with alkali was 0.6%.

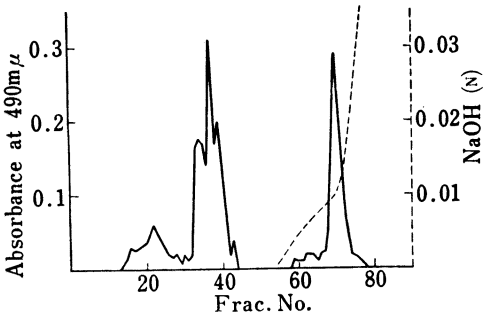

Fig. 2. Chromatogram on DEAE-cellulose

Gel-permeation Chromatography on Sephadex Columns——Sephadex G-100 and G-200 (Pharmacia Co.) were treated with hot water for several hours and washed repeatedly with water, followed by washing with 0.1 M ammonium formate. After preparation of a column (2.6×96 cm), the elution was carried out by ascending method using a Mariotte flask and a flow adaptor with 0.1 M ammonium formate as an eluant. Fractions were collected at 20 ml in the case of Sephadex G-100 column and at 5 ml in the case of Sephadex G-200 column, and analyzed by phenol–sulfuric acid method.[4] The result of the application of the alkali eluate, obtained from DEAE-cellulose column, to Sephadex G-100 column is shown in Fig. 3. Fig. 4 shows the result of the gel-permeation chromatography of plantasan on Sephadex G-200.

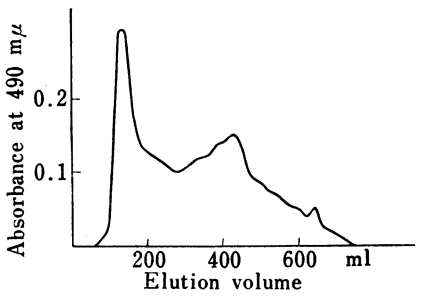

Fig. 3. Chromatogram of Alkali Eluate on Sephadex G-100

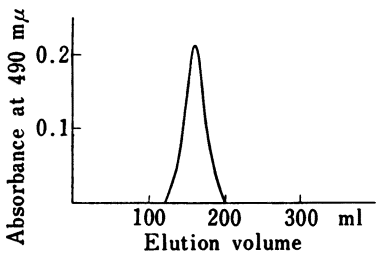

Fig. 4. Chromatogram of Plantasan on Sephadex G-200

Glass-Fiber Paper Electrophoresis——Electrophoresis was carried out with Tōyō GB60 glass-fiber (12×24 cm long) and alkaline borate buffer of pH 12 (0.1 N NaOH: 0.025 M borax, 1:1) at the condition of 240 volt for 2 hr. Samples were applied in line at 8 cm from the anode, and moved toward the cathode.

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The inside of the apparatus was cooled with dry ice. *p*-Anicidine sulfuric acid reagent[5] was used for detection. Plantasan gave one spot at a distance of 1.9 cm from the origin. Distance moved by D-glucose was 9.7 cm.

Thin-Layer Chromatography of Hydrolysate——Polysaccharides were hydrolyzed with 1 N sulfuric acid at 100° for 6 hr, then neutralized with barium carbonate. The filtrates were passed through small column of Dowex 50W-X8 (H^+) for the removal of barium ion. Avicel SF cellulose (20 g) were mixed with water (90 ml) in a homogenizer and glass plates (20 × 20 cm) were coated with 0.25 mm thick layer by the use of an applicator. The plates were used after standing overnight at room temperature. The samples were spotted in line at 1.5 cm from an edge and the solvents were allowed to ascend to a height of 10 cm from starting point at 20°. The following solvent systems were used; A, AcOEt: pyridine: AcOH: H_2O (5:5:1:3, by vol.); B, AcOEt: pyridine: AcOH: H_2O (30:10:1:6, by vol.); C, C_6H_5OH: 1% NH_4OH (2:1, by vol.). The component sugars were revealed with silver nitrate reagent[6] and *p*-anisidine reagent.[7] Table I shows *Rf* values of sugars in the three solvent systems.

Table I. *Rf* Values of Component Sugars

	Solvent A	Solvent B	Solvent C
L-Rhamnose	0.74	0.59	0.55
D-Xylose	0.60	0.35	0.38
L-Arabinose	0.54	0.26	0.46
D-Galactose	0.46	0.12	0.35
D-Galacturonic acid	0.20	0.03	0.11

Determination of Component Sugars——Galacturonic acid was determined by carbazole method.[8] Rhamnose was estimated by thioglycolic acid method.[9] Galactose was measured by cystein sulfuric acid method (secondary reaction).[10] For the quantitative analyses of xylose and arabinose, gas chromatography[11] of the trifluoroacetate of reduction product of hydrolysate was carried out using Hitachi model F6D with 2% XF1105 on Gas-Chrom P (2 m long glass column) at 140° with a flow of 40 ml per min of nitrogen. The values of pentose and hexuronic acid were also supported by the result of orcinol method.[12] The present study showed that plantasan contains 57.5% of D-xylose, 12.1% of L-arabinose, 20.2% of D-galacturonic acid, 8.4% of L-rhamnose and 1.9% of D-galactose (all percentages are for anhydrosugars).

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