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Plant Mucilages. II.[1] Isolation and Characterization of a Mucous Polysaccharide, "Odoratan," from *Polygonatum odoratum* var. *japonicum* Rhizomes

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A mucous polysaccharide, named odoratan, has been isolated from the rhizomes of *Polygonatum odoratum* Druce var. *japonicum* Hara. It was homogeneous on gel chromatography and glass-fiber paper electrophoresis. The component carbohydrates of it were D-fructose, D-mannose, D-glucose and D-galacturonic acid, and the molar ratio of them was 6:3:1:1.5. Component D-fructose was almost liberated by the digestion with β-fructofuranosidase.

The rhizome of *Polygonatum odoratum* Druce var. *japonicum* Hara (=*Polygonatum odoratum* Druce var. *pluriflorum* Ohwi) has been used as a crude drug for the purpose of analeptic. We wish to report the isolation of a mucous polysaccharide from the rhizome of this plant and its properties are also described in the present paper. On the constituents of the rhizome of *Polygonatum odoratum* Druce (=*Polygonatum officinale* All.), presences of a mucilage[3] and an imino acid[4] have been reported until now. Gaal[3] has concluded that the mucilage is composed of D-fructose (81.7%), D-glucose and L-arabinose. But the new mucous polysaccharide obtained by us has different properties from it even in respect of component sugars.

The fresh rhizomes were extracted with hot methanol, then the residue was extracted with hot water. The methanol extract contains glucose, fructose and sucrose. The crude mucilages were precipitated from the water extract by addition of ethanol. The supernatant contains polysaccharides and oligosaccharides composed of fructose and glucose. The solution of the precipitate was applied to a DEAE-cellulose (acetate form) column, and a mucous polysaccharide was obtained from the eluate with water.

The polysaccharide was homogeneous on gel filtration with Sephadex G-200 and gave one spot on glass-fiber paper electrophoresis in alkaline borate buffer. The name "odoratan" is proposed for the polysaccharide. It showed a negative specific rotation ($[\alpha]_D^{20}$—29.2° in H_2O, c=0.3).

Although small amount of the other polysaccharide fraction was obtained from the DEAE-cellulose column with a gradient elution of sodium acetate solution, the result of glass-fiber paper electrophoresis showed that the fraction is a mixture of two polysaccharides. The outline of the fractionation is shown in Chart 1.

It was shown that the component sugars of odoratan are fructose, mannose, glucose and galacturonic acid by means of cellulose thin-layer chromatography of the hydrolysate and gas-liquid chromatography of its trimethylsilyl derivative. The fraction B-2 of Chart 1 contains rhamnose, xylose, arabinose and galactose in addition to fructose, mannose, glucose, and galacturonic acid as its component sugars.

1) Part I: M. Tomoda and M. Uno, *Chem. Pharm. Bull.* (Tokyo), **19**, 1214 (1971).
2) Location: *6-3, Shibakoen, Minato-ku, Tokyo.*
3) B. Gaal, *Ber. Ungar. Pharm. Ges.*, 3, 133 (1927) [*Chem. Abstr.*, 23, 1929 (1929)].
4) *a*) A.I. Virtanen and P. Linko, *Acta Chem. Scand.*, **9**, 551 (1955); *b*) A.I. Virtanen, *Nature*, **176**, 984 (1955).

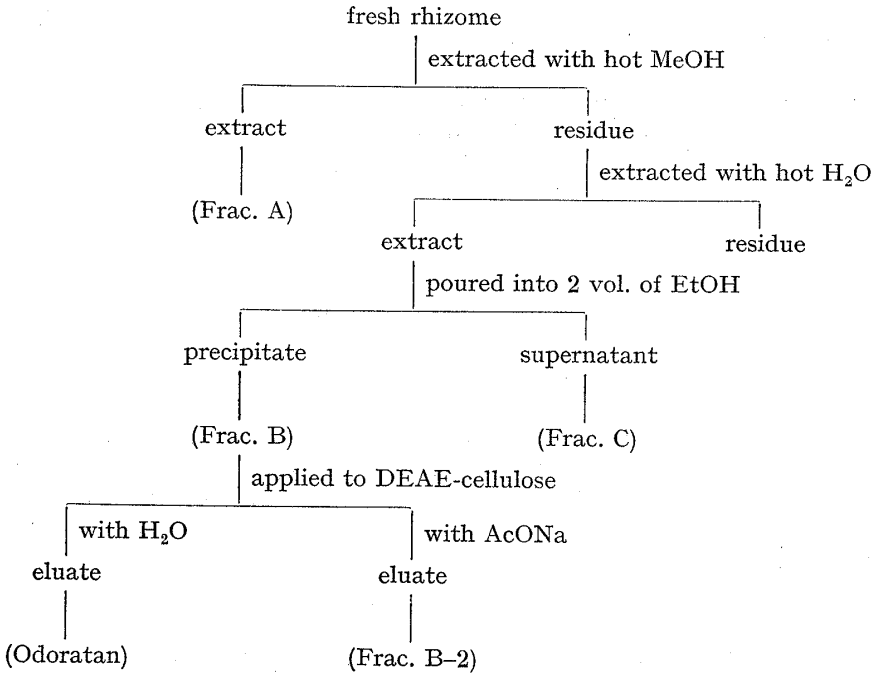

Chart 1. Isolation and Fractionation of Water-soluble Constituents

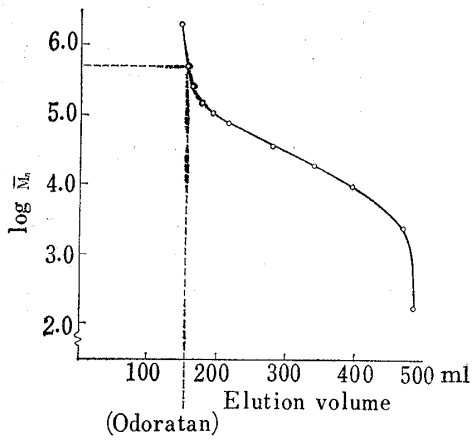

Fig. 1. Plot of Elution Volume against log $\bar{M}n$ for Dextran Fractions on Sephadex G-200 with 0.1M Ammonium Formate

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 odoratan thus estimated was *ca.* 500000. This value must be regarded as approximate, but it was supported by the result of molecular-sieve chromatography of an enzymatic degradation product obtained from odoratan.

Quantitative determinations of the sugar components of odoratan showed that the molar ratio of them was as follows; fructose: mannose: glucose: galacturonic acid was about 6: 3: 1: 1.5. The water solution of odoratan gave the intrinsic viscosity value of 2.2 at 20°. On the other hand, the fraction B of Chart 1, the source of odoratan, gave the intrinsic viscosity value of 3.5 in its water solution. This result suggests the presence of the other unknown mucous substance in the fraction B. Fractions A, B-2 and C of Chart 1 showed very low or no viscosity in their water solution.

By means of the digestion with β-fructofuranosidase (β-D-fructofuranoside fructohydrolase, 3. 2. 1. 26), 93.6% of fructose was liberated from odoratan. And the ketohexose was the single carbohydrate of low molecular weight produced by the enzymatic action. The residue after liberation of fructose was homogeneous on molecular-sieve chromatography and its molecular weight was estimated as *ca.* 250000.

As the result of periodate oxidation, 0.715 mole of periodate per one mole of the average component anhydrosugar of odoratan was consumed with 0.037 mole of formic acid liberation.

From the results of enzymatic degradation and periodate oxidation, it is conceivable that fructofuranose composes linear units, and on the average, odoratan contains 27 units per end group. This presumption is due to the fact that the presence of 1→6 linear linkage

is denied by very low formic acid liberation. The detail of the structure of odoratan will be discussed in following papers.

Experimental

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

Isolation of Odoratan——The material was obtained in September of 1970 from the plants cultivated in Saitama prefecture. The fresh rhizomes (400 g), which contain 67.9% of water, were crushed, then extracted with hot methanol (1600 ml) for 1 hr. After suction filtration, the extraction was similarly repeated. The extracts were combined and concentrated to 20 ml, followed by lyophilization. Pale brown powder (46.5 g) was obtained. After extraction with methanol, the residue was extracted with hot water (1600 ml) for 1 hr. Suction filtration was carried out through a sheet of glass wool, and the filtrate was poured into twofold volume of ethanol, then centrifuged. The precipitate was treated with absolute ethanol, and dried *in vacuo*. White powder (3.6 g) was obtaind. The supernatant was concentrated and lyophilized. Pale yellow powder (18.4 g) was obtained.

The first precipitate (Frac. B of Chart 1, 314 mg) from water extract was dissolved in water (30 ml) and applied to a column (2.6×80 cm) of DEAE-cellulose (Brown Co.). DEAE-cellulose was previously treated with 0.5N sodium hydroxide and the excess of alkali was removed by rinsing with water. Then the ion exchanger was treated with 0.5M sodium acetate and the excess of salt was removed with water. The column was eluted with water, followed by gradient elution with sodium acetate. Fractions of 20 ml were collected and analyzed by phenol-sulfuric acid method.[5] The result was shown in Fig. 2. The eluate with water was lyophilized and the polysaccharide "odoratan" was obtained as white powder. Yield, 60.5% from Frac. B. The eluate with sodium acetate was dialyzed for two days and the solution was passed through a mixed bed resin column (0.5×2 cm) containing Dowex 50W (H^+) and Dowex 2 (OH^-). After concentration, ethanol was added and the precipitate was lyophilized. Yield, 3.8% from Frac. B.

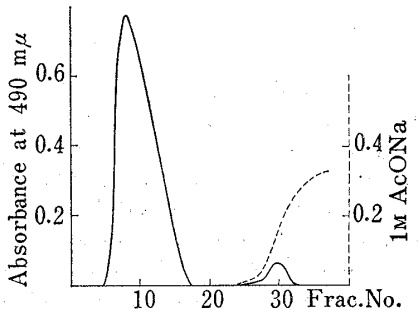

Fig. 2. Chromatogram on DEAE-cellulose

Glass-Fiber Paper Electrophoresis——Electrophoresis was carried out with Whatman GF 83 glass-fiber (12×38 cm long) and alkaline borate buffer of pH 12 (0.1N NaOH: 0.025M borax, 1:1) at the condition of 380 volt for 2 hr. Samples were applied in line at 13 cm from the anode, and moved toward the cathod. The inside of the apparatus was cooled with dry ice. *p*-Anisidine-sulfuric acid reagent[6] was used for detection. Odoratan gave one spot at a distance of 13.7 cm from the origin, and Frac. B-2 of Chart 1 gave two spots at distances of 13.2 cm and 7.8 cm. Distance moved by D-glucose was 15.3 cm.

Gel Chromatography on a Sephadex Column——A column (2.6×96 cm) of Sephadex G-200 (Pharmacia Co.) was prepared and the elution was carried out as described in the previous report of this series. Fractions were collected at 5 ml and analyzed by phenol-sulfuric acid method.

Qualitative Analyses of Hydrolysate——For perfect hydrolysis of polysaccharides, samples were heated with 2N sulfuric acid at 100° for 6 hr, then neutralized with barium carbonate. On the other hand, mild hydrolysis was performed with 0.5N sulfuric acid at 60° for 2 hr for the purpose of the detection of D-fructose. After neutralization, filtrates were passed through small columns of Dowex 50W (H^+) for the removal of barium ion.

Thin-layer chromatography using Avicel SF cellulose was carried out in the same way as the previous report of this series. Two solvent systems were used: A, AcOEt: pyridine: AcOH: H_2O (5:5:1:3, by vol.); B, PhOH: 1% NH_4OH (2:1, by vol.). The component sugars were revealed with silver nitrate reagent,[7] diphenylamine-aniline reagent,[8] naphthoresorcinol-phosphoric acid reagent[9] and benzidine reagent.[10]

For analysis by gas-liquid chromatography, trimethylsilyl derivatives of the hydrolysates were prepared by the method of Sweeley, *et al.*,[11] followed by removal of reagents as reported by Yamakawa, *et al.*[12] The

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11) C.C. Sweeley, R. Bentley, M. Makita and W.W. Wells, *J. Am. Chem. Soc.*, **85**, 2497 (1963).
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chloroform solution was applied to Hitachi model F6D gas chromatograph with hydrogen flame ion detector by the use of a column (0.3 cm inner diameter × 1 m long U-shape stainless steel) packed with 3% SE 52 on Chromosorb W (80 to 100 mesh) and with a flow of 20 ml per min of nitrogen. The programmed temperature was increased 2.5° per min from 130° to 180°. Table I shows *Rf* values in TLC and retention times in GLC of component sugars. In this condition of GLC, appearances of galacturonic acid peaks were hindered by the presence of aldohexose in the hydrolysate of odoratan.

Table I. *Rf* Values and Retention Times of Component Sugars

	TLC (*Rf*)		GLC (t_R)
	Solvent A	Solvent B	
D-Fructose	0.53	0.45	11.3
D-Mannose	0.52	0.39	11.0
D-Glucose	0.46	0.34	14.0, 16.8
D-Galacturonic acid	0.14	0.11	13.3, 16.3, 18.0
Hydrolysate ($2N$ H_2SO_4, 100°, 6 hr)	0.52 0.46 0.14	0.39 0.34 0.11	11.0 14.0 16.8
Hydrolysate ($0.5N$ H_2SO_4, 60°, 2 hr)	0.53	0.45	11.3

Determination of Component Sugars——D-Fructose was determined by resorcinol method.[13] D-Galacturonic acid was estimated by orcinol method.[14] For the quantitative analyses of D-glucose and D-mannose, hydrolysis was done with 2N sulfuric acid at 100° for 3 hr. D-Xylose was used as an intrinsic standard, and gas–liquid chromatography[15] of the trifluoroacetate of reduction product of the hydrolysate was carried out by the use of Hitachi model F6D with 2% XF 1105 on Gas-Chrom P (80 to 100 mesh, 0.4 cm × 2 m long spiral glass column) at 140° with a flow of 40 ml per min of nitrogen. The results revealed that odoratan contains 51.9% of D-fructose, 25.0% of D-mannose, 8.4% of D-glucose and 14.8% of D-galacturonic acid (All percentages are for anhydrosugars.)

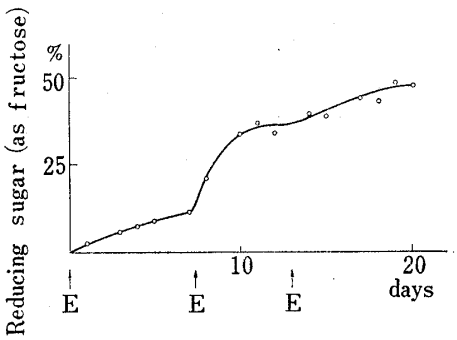

Fig. 3. Rise of Reducing Activity by Enzymatic Degradation

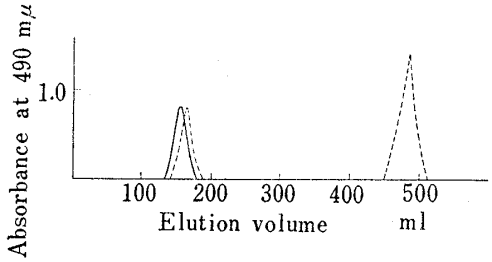

Fig. 4. Chromatogram on Sephadex G-200

——— : Odoratan
····· : Enzymatic Degradation Products

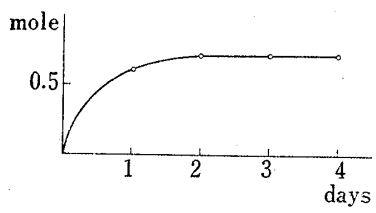

Fig. 5. Periodate Oxidation

Enzymatic Degradation——Odoratan (100 mg) was dissolved in 5 ml of water and 2 mg of β-fructofuranosidase (from yeast, Boehringer Co.) was added. The solution was incubated at 40°, and the enzyme (2 mg each) was further added in twice after 7 days and 13 days. After varying periods of time, the hydrolysate was analyzed for reducting activity by the method of Park and Johnson.[16] The liberated monosaccharide was detected by cellulose thin-layer chromatography as described previously. The rise of reducting activity was shown in Fig. 3, and the results of gel chromatography of odoratan and its enzymatic degradation products were shown in Fig. 4.

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14) A.H. Brown, *Arch. Biochem.*, **11**, 269 (1946).
15) T. Imanari, Y. Arakawa and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **17**, 1967 (1969).
16) J. T. Park and M. J. Johnson, *J. Biol. Chem.*, **181**, 149 (1949).

Periodate Oxidation——The sample (150 mg) was oxidized with 0.1M sodium metaperiodate (30 ml) at room temperature in a dark place. The periodate consumption was measured by a spectrophotometric method.[17] The oxidation was completed after three days (Fig. 5), then the formic acid liberation was measured by a titration with 0.01N NaOH.

17) J. S. Dixon and D. Lipkin, *Anal. Chem.*, **26**, 1092 (1954).