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Plant Mucilages. V.[1] Isolation and Characterization of a Mucous Polysaccharide, "Falcatan," from *Polygonatum falcatum* Rhizomes

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The rhizome of *Polygonatum falcatum* A. Gray has been used as a crude drug for the purpose of analeptic, but no report on the constituents of this rhizome has been published up to the present time. We have now isolated a mucous polysaccharide from this material, and its properties are described in this paper.

The fresh rhizomes were extracted with hot methanol, followed by extraction of the residue with hot water. The methanol extract contains fructose, glucose, sucrose and oligosaccharides composed of fructose and glucose. The crude mucilages were precipitated from the water extract by the addition of ethanol. The supernatant contains polysaccharides composed of fructose, glucose and galacturonic acid. 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. Thus the outline of the fractionation is similar to the case of odoratan[3] (Chart 1).

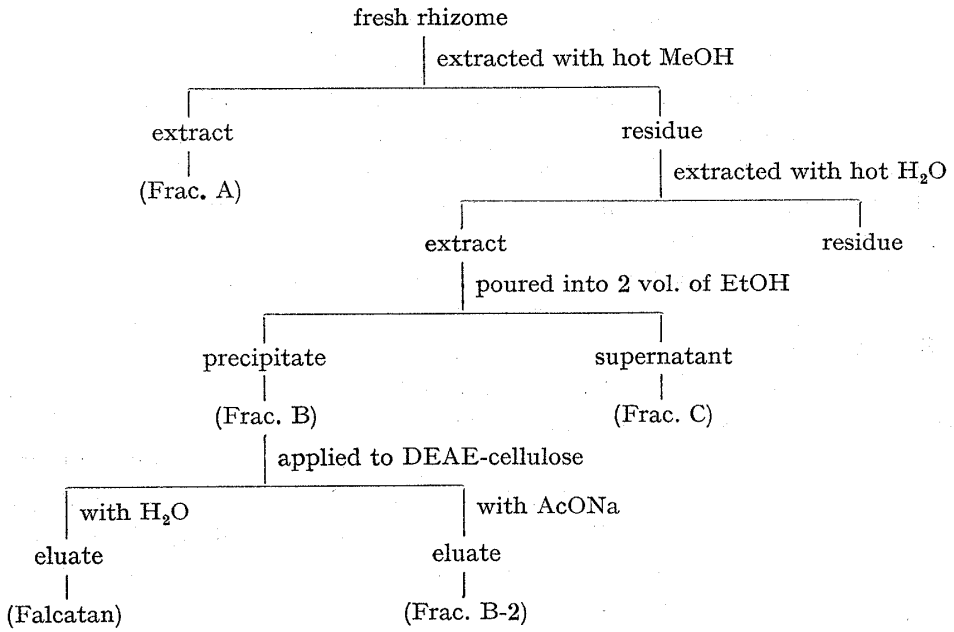

Chart 1. Isolation and Fractionation of Constituents

The polysaccharide gave one spot on glass-fiber paper electrophoresis in alkaline borate buffer and was homogeneous on gel chromatographies with Sephadex G-200 and Sepharose 4B. The name "falcatan" is proposed for the polysaccharide. It showed a negative specific

1) Part IV: M. Tomoda, S. Nakatsuka, and E. Minami, *Chem. Pharm. Bull.* (Tokyo), **20**, 953 (1972).
2) Location: *1-5-30, Shibakōen, Minato-ku, Tokyo, 105, Japan.*
3) M. Tomoda, Y. Yoshida, H. Tanaka, and M. Uno, *Chem. Pharm. Bull.* (Tokyo), **19**, 2173 (1971).

rotation ($[\alpha]_D^{21}$ $-29.0°$ in H_2O, $c=0.5$). Its solution in water gave the intrinsic viscosity value of 2.75 at 25°.

As the component sugars of falcatan, fructose, mannose, glucose, and galacturonic acid were identified by means of cellulose thin-layer chromatography of the hydrolysate and gas-liquid chromatography of its trimethylsilyl derivative. Quantitative determinations of them showed that the molar ratio was as follows; fructose: mannose: glucose: galacturonic acid was about 25: 10: 5: 1.

The average molecular weight of falcatan estimated from the calibration curve,[3] which is obtained by the gel chromatography of standard dextran fractions of known molecular weights on Sepharose 4B, was *ca.* 420000. The measurement of osmotic pressure gave the value of 410000 as the molecular weight of falcatan.

Less amount of the other polysaccharide fraction was obtained from the DEAE-cellulose column by the elution with sodium acetate solution, and this fraction contains galactose, arabinose, xylose, rhamnose, and galacturonic acid as its component sugars. But no fraction showed evident mucosity in aqueous solution except falcatan.

By means of the digestion with β-fructofuranosidase, 95.3% of fructose was liberated from falcatan. And fructose was the single carbohydrate of low molecular weight produced by the enzymic action. The residue after liberation of fructose was homogeneous on gel chromatography with Sephadex G-200 and its molecular weight was estimated as *ca.* 170000 from the calibration curve.[3] This result provided the clear evidence that the estimated fructose in falcatan exists as its component.

As the result of periodate oxidation, 0.81 mole of periodate per one mole of the average component anhydro sugar of falcatan was consumed with 0.03 mole of formic acid liberation. The periodate-oxidized polysaccharide was treated by the Smith procedure,[4] and the analysis of the hydrolysate of the reduction product revealed the presences of glycerol, erythritol and mannose. Quantitative determination of them by gas-liquid chromatography of trimethylsilyl derivatives showed that their molar ratio was about 16: 2: 1.

The formation of a large quantity of glycerol by Smith degradation and the result of the enzymic degradation suggest that fructose exists as 2→1 or 2→6 linked ketohexofuranose residue. The presences of 1→2 and 1→4 linked aldohexopyranose residues were also indicated from the result of Smith degradation, because erythritol was produced in addition to a large quantity of glycerol. It is probable that a part of mannose residues occupies branching positions in falcatan, although the possibility of the presence of some of mannose residues having 1→3 linkage is unable to be denied yet.

As already described in the former report,[3] a mucous polysaccharide, odoratan, has been isolated from the rhizomes of *Polygonatum odoratum* Druce var. *japonicum* Hara and its properties were investigated in this laboratory. Odoratan has the same component sugars as falcatan, although molar ratio of them differs from that of the latter. Both polysaccharides show similar viscosity and have approximately the same molecular weight and it is interesting that they contain many fructose residues as a component. The detail of the structure will be reported in following papers.

Experimental

Solutions were evaporated at or below 40° with rotary evaporators under reduced pressure. Specific rotation was measured by the use of JASCO model DIP-SL automatic polarimeter. Gas-liquid chromatography was carried out by the use of Hitachi model 063 gas chromatograph equipped with hydrogen flame ion detector.

Isolation of Falcatan——The material was obtained in October of 1971 from the plants cultivated in Saitama prefecture. The fresh rhizomes (400 g), which contain 73.8% of water, were crushed, then ex-

4) I.J. Goldstein, G.W. Hay, B.A. Lewis, and F. Smith, "Methods in Carbohydrate Chemistry," No. V, Academic Press, New York and London, 1965, p. 361.

tracted with hot methanol (1600 ml) for 1 hr. After suction filtration, the extraction was similarly repeated. The extracts were combined and concentrated to 50 ml, followed by lyophilization. Yellow powder (49.8 g) was obtained. After extraction with methanol, the residue was extracted with hot water (1600 ml) for 1 hr twice. After suction filtration, the combined filtrate was poured into two volumes of ethanol, then filtered. The filtrate was concentrated and lyophilized. Pale yellow powder (17.1 g) was obtained. The precipitate was treated with absolute ethanol and acetone, then dried *in vacuo*. White powder (5.8 g) was obtained (Frac. B of Chart 1).

Frac. B (1 g) was dissolved in water (50 ml) and applied to a column (4.5×60 cm) of DEAE-cellulose (Brown Co.). DEAE-cellulose was used as acetate form by the previous treatments with sodium hydroxide and sodium acetate as described in the preceding report.[3] The column was eluted with water, followed by elution with 1M sodium acetate. Fractions of 20 ml were collected and analyzed by phenol-sulfuric acid method.[5] The result was shown in Fig. 1. The eluate with water was lyophilized and the polysaccharide "falcatan" was obtained as white powder. Yield, 55.9% 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^-). The eluate was concentrated and lyophilized. Yield, 27.7% from Frac. B.

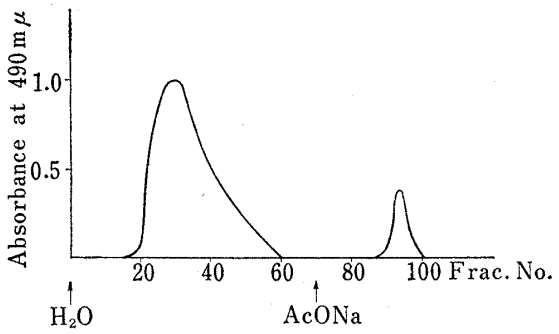

Fig. 1. Chromatogram on DEAE-cellulose

Glass-Fiber Paper Electrophoresis——Electrophoresis was carried out with Whatman GF 83 glass-fiber and alkaline borate buffer of pH 12 in the same manner as the preceding report[3] of this series. Falcatan gave one spot at a distance of 13.6 cm from the origin toward the cathod. Distance moved by standard glucose was 16.0 cm.

Gel Chromatographies on Sephadex and Sepharose Columns——A column (2.6×92 cm) of Sephadex G-200 (Pharmacia Co.) was prepared and the elution was carried out by ascending method as described in the first report[6] of this series. Fractions were collected at 3 ml and analyzed by phenol-sulfuric acid method. Elution volume of falcatan peak was 163 ml, and it was 178 ml in the enzymic degradation product. In the case of Sepharose 4B (Pharmacia Co.), a column (2.6×96 cm) was prepared and the elution was carried out by similar ascending method with 0.02M sodium phosphate buffer (pH 7.0) as an eluant. Fractions were collected at 5 ml and analyzed by phenol–sulfuric acid method. Elution volume of falcatan peak was 275 ml.

Qualitative Analyses of Component Sugars——Samples were heated with 2N sulfuric acid at 100° for 6 hr for perfect hydrolysis, and heated with 0.5N sulfuric acid at 60° for 2 hr for the detection of fructose. After neutralization with barium carbonate, 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 with two solvent systems: A, AcOEt: pyridine: AcOH: H_2O (5: 5: 1: 3, by vol.); B, C_6H_5OH: 1% NH_4OH (2: 1, by vol.). Component sugars were revealed with silver nitrate reagent,[7] benzidine reagent[8] and naphthoresorcinol–phosphoric acid reagent.[9]

On the other hand, trimethylsilyl derivatives of the hydrolysates were prepared by the method of Sweeley, *et al.*,[10] and they were applied to gas chromatograph by the use of a column (0.3 cm inner diameter×2 m long spiral 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 3° per min from 130° to 220°. Table I shows *Rf* values in TLC and retention times in GLC of component sugars.

Determination of Component Sugars——Fructose was determined by resorcinol method.[11] Galacturonic acid was estimated by carbazole method.[12] For the quantitative analyses of mannose and glucose, the sample was hydrolyzed with 2N sulfuric acid at 100° for 3 hr, then reduced with sodium borohydride, and the trifluoroacetate[13] of reduction product was applied to gas chromatograph by the use of a column (0.4 cm

5) M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith, *Anal. Chem.*, **28**, 350 (1956).
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12) Z. Dische, *J. Biol. Chem.*, **167**, 189 (1947).
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Table I. *Rf* Values and Retention Times of Component Sugars

	TLC (*Rf*)		GLC (t_R)
	Solvent A	Solvent B	
Fructose	0.53	0.45	17.0
Mannose	0.52	0.38	16.5
Glucose	0.48	0.32	19.5, 22.3
Galacturonic acid	0.16	0.11	
Hydrolysate (2N H_2SO_4, 100°, 6 hr)	0.52	0.38	16.5
	0.48	0.32	19.5
	0.16	0.11	22.3
Hydrolysate (0.5N H_2SO_4, 60°, 2 hr)	0.53	0.45	17.0

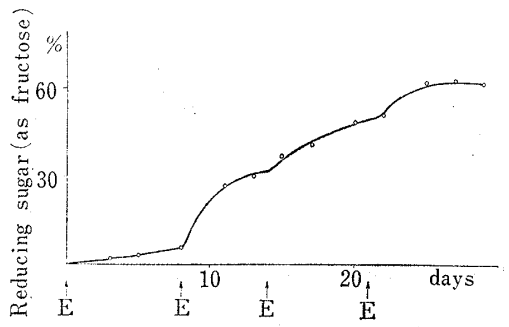

Fig. 2. Rise of Reducing Activity by Enzymic Degradation

inner diameter × 2 m long spiral glass) packed with 2% XF 1105 on Gas-Chrom P (80 to 100 mesh) at 140° with a flow of 30 ml per min of nitrogen. Xylitol was used as an internal standard. The results revealed that falcatan contains 59.5% of fructose, 24.3% of mannose, 12.2% of glucose and 2.6% of galacturonic acid (All percentages are for anhydrosugars).

Measurement of Osmotic Pressure——Osmotic pressure was measured at 60° by the use of Knauer Electronic Membrane Osmometer. The sample was dissolved in water, and 0.4, 0.3, 0.2, and 0.1% solution were used.

Enzymic Degradation and Measurement of Liberated Fructose——These were carried out in the same manner as the case of odoratan,[3] but β-fructofuranosidase (Boehringer Co.) was further added in three times after 8 days, 14 days and 21 days from the first addition. The increase of reducing activity was shown in Fig. 2.

Periodate Oxidation and Smith Degradation——Falcatan (20 mg) was oxidized with 0.05M sodium metaperiodate (10 ml) at room temperature in a dark place. The periodate consumption was measured by a spectrophotometric method.[14] The oxidation was completed after four days, then formic acid liberation was measured by a titration with 0.01N NaOH. The solution was reduced with sodium borohydride (0.1 g) at 5° for 16 hr, then acetic acid was added up to pH 5, and the reaction mixture was passed through columns of Dowex 50W (H^+, 2×18 cm) and Dowex 44 (OH^-, 2×9 cm). The eluate and washing were combined and evaporated *in vacuo* to dryness. The residue was heated with 0.2N hydrochloric acid at 100° for 6 hr, then evaporated *in vacuo* and further treated three times with methanol followed by evaporation for the removal of acid.

Analyses of Smith Degradation Products——The hydrolysate (1 mg) was dissolved in pyridine (0.1 ml) containing trimethylolpropane (0.25 mg) as an internal standard, then subjected to trimethylsilylation by addition of hexamethyldisilazane (0.02 ml) and trimethylchlorosilane (0.01 ml). The product was applied to gas chromatography and determined.

Table II. Retention Times and *Rf* Values of Smith Degradation Products

	GLC (t_R)	TLC (*Rf*)	
		Solvent C	Solvent D
Glycerol	19.5	0.47	0.66
Erythritol	26.0	0.33	0.49
Mannose	32.9, 35.0	0.25	0.18
Trimethylolpropane (internal standard)	23.0		

14) *a*) J.S. Dixon and D. Lipkin, *Anal. Chem.*, **26**, 1092 (1954); *b*) G.O. Aspinall and R.J. Ferrier, *Chem. Ind.*, **1957**, 1216.

GLC: Column, 5% SE 30 on Chromosorb G (80 to 100 mesh) (0.3 cm inner diameter × 2 m long stainless steel); programmed column temperature, increase in 5° per min from 60° to 260°; carrier gas, N_2 (30 ml per min).

On the other hand, thin-layer chromatography of the hydrolysate using Avicel SF cellulose was carried out. Two solvent systems were used; C, AcOEt: pyridine: H_2O (10: 4: 3, by vol.); D, AcOEt: AcOH: HCOOH: H_2O (18: 3: 1: 4, by vol.). The products were detected with periodate–permanganate–benzidine reagent.[15] Table II shows retention times of trimethylsilyl derivatives in GLC and *Rf* values in TLC.

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An Unequivocal Synthesis of 1-Methylthio-7-chlorophenazine[1]

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Recent article[1] from our laboratory has described that while irradiation of N-acetyl-4-chloro-2-nitro-2′-methylthiodiphenylamine (I) gives 1-methylthio-7-chlorophenazine-5-oxide (IV) which is readily reduced to 1-methylthio-7-chlorophenazine (V), reduction of I with triethyl phosphite followed by autooxidation leads to the formation of isomeric 1-methylthio-8-chlorophenazine (III). This interesting observation makes it possible to predict that the cyclization of I to dihydrophenazine (II) by triethyl phosphite could involve a novel molecular rearrangement as shown in Chart 1. The structure of V has been assigned on the basis of physicochemical data and mechanistic consideration[3] of the photocyclization of I leading to IV.

In this paper, we now describe an unequivocal synthesis of V, which also confirms the structure of III.

Phenazines have frequently been prepared[4] by the condensation of *o*-benzoquinones with *o*-phenylenediamines, the thermal condensation of catechols with *o*-phenylenediamines, or the Wohl-Aue condensation of nitrobenzenes and anilines. These procedures, however, are not adequate for the synthesis of V because of unaccessible starting materials and low yields with several side products. Reaction conditions for cyclizations of 2-nitrodiphenylamines to phenazines have been investigated by several groups of workers,[5] but their results are not satisfactory for synthetic purposes. More recently, Cross and co-workers[6] have reported that novel cyclization of 2-nitrodiphenylamines to phenazines and their N-oxides in both acidic

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6) B. Cross, D.J. Williams, and R.E. Woodall, *J. Chem. Soc.* (*C*), **1971**, 2085.