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### Water-soluble Carbohydrates of *Ophiopogon* Tuber. III.<sup>1)</sup> Isolation and Characterization of a new Inulin-type Fructan\*

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The water-soluble oligosaccharides and polysaccharides composed of D-fructose and D-glucose are the main constituent of the tuberous roots of *Ophiopogon japonicus* KER-GAWLIER var. *genuinus* MAXIMOWICZ,<sup>2)</sup> and the properties of three oligosaccharides have already been described in the previous paper in this series.<sup>1)</sup> We have now isolated the other polysaccharide from the water extract of the material in good yield, and its property and structure are reported in the present paper.

The process for the isolation of the substance was, on the whole, similar to the case of the other oligosaccharides. The water extract of the material was applied to a charcoal column, and seven fractions were obtained by elution with water and stepwise increments of ethanol.

It is known that all fructans are very susceptible to hydrolysis. In consideration of this property, the extraction with water was carried out at 40°. But the yields of the fractions obtained from a charcoal column were generally similar to those in the former case<sup>1)</sup> which the extraction was done with hot water, so it is conceivable that no noticeable depolymerization of fructans has taken place under the extraction by heating with water.

The fraction eluted with 25% ethanol was applied to a column of Sephadex G-25. The repeated gel chromatography gave a new non-reducing polysaccharide which showed a single spot on multi-developed cellulose thin-layer chromatography (TLC). It was obtained as a white powder,  $[\alpha]_D^{25} -43.2^\circ$  (H<sub>2</sub>O, *c*=2). The value of 3440 was obtained as its molecular weight by the use of a vapor pressure osmometer.

Gas-liquid chromatography (GLC) of trimethylsilylated derivative of the methanolysate and TLC of the hydrolysate of the polysaccharide revealed that the component sugars are D-fructose and D-glucose, and in addition to these data, the result of quantitative determination of them provided the conclusion that the polysaccharide is composed of twenty fructose units and one glucose unit.

As the result of periodate oxidation, 0.97 mole of periodate per one mole of the component anhydrosugar unit of the polysaccharide was consumed with 0.06 mole of formic acid liberation. The periodate-oxidized sample was reduced with sodium borohydride and the analysis of the mild hydrolysate of the product showed the presence of glycerol and no appearance of component hexose.

\* 本報告は *Chem. Pharm. Bull.* (Tokyo), **21**, 659 (1973) に発表.

1) Part II: M. Tomoda and S. Katō, *Chem. Pharm. Bull.* (Tokyo), **16**, 113 (1968).

2) M. Tomoda and S. Katō, *Shōyaku-gaku Zasshi*, **20**, 12 (1966).

Methylation of the sample was performed with barium oxide and dimethyl sulfate in dimethylformamide and dimethyl sulfoxide. After mild hydrolysis and methanolysis of the methylated product, the presences of methyl 3,4,6-trimethyl  $\text{D}$ -fructoside, methyl 1,3,4,6-tetramethyl  $\text{D}$ -fructoside and methyl 2,3,4,6-tetramethyl  $\text{D}$ -glucoside were proved by GLC. The methylation was also done with sodium hydride and methyl iodide in dimethyl sulfoxide, and the gas-chromatographic analysis of the methanolysate of the product showed the same result. From these results, the structure illustrated in Chart 1 could be proposed to the new fructan.

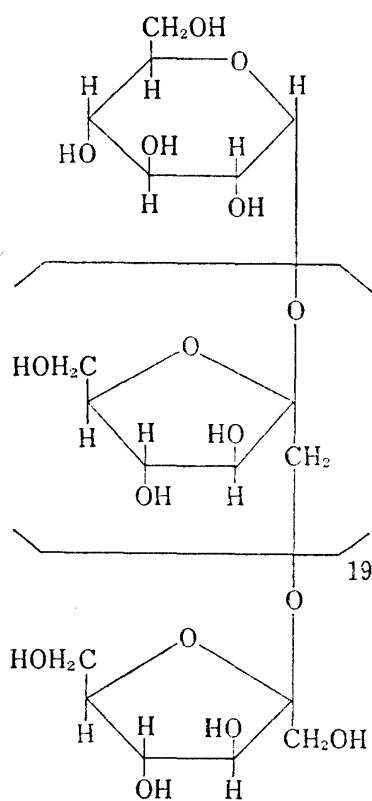


Chart 1

Many fructans have been found as reserve carbohydrates in various plants and they differ from each other in structure and in molecular weight. They are divided into two main groups, that is, inulin group and phlean group. The fructan reported in this paper is a typical inulin-type polysaccharide but its molecular weight differs distinctly from the known inulin. The eluates with 15% and 20% ethanol obtained from the charcoal column chromatography also gave methyl glycosides of 3,4,6-trimethyl  $\text{D}$ -fructose, 1,3,4,6-tetramethyl  $\text{D}$ -fructose and 2,3,4,6-tetramethyl  $\text{D}$ -glucose as the products of methanolysis after methylation. The other trimethyl, dimethyl and monomethyl  $\text{D}$ -fructoside were not found in the products. Therefore, it is able to conclude that the water-soluble oligosaccharides and polysaccharides of *Ophiopogonis Tuber* belong to inulin-type fructans having various molecular weights.