Notes

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Water-soluble Carbohydrates of Ophiopogonis Tuber. III.[1] 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-Gawler var. *genuinus* Maximowicz,[3] and the properties of three oligosaccharides have already been described in the previous paper in this series.[1] 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[1] which the extraction was done with hot water. It is thus 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, $[\alpha]_D^{18}$ −43.2° (H_2O, 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[4] showed the presence of glycerol and no appearance of component hexose.

Methylation of the sample was performed with barium oxide and dimethyl sulfate in dimethylformamide and dimethyl sulfoxide.[5] After mild hydrolysis and methanolysis of the methylated product, the presences of methyl 3,4,6-trimethyl D-fructoside, methyl 1,3,4,6-

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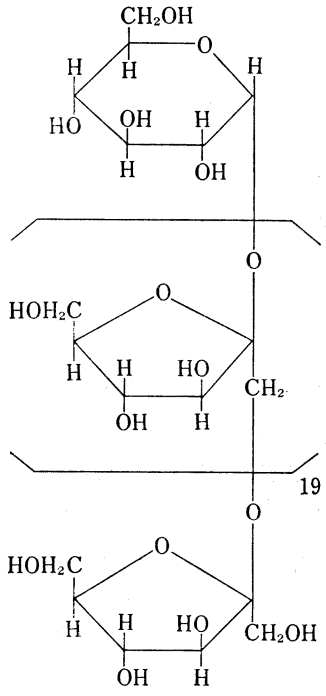

Chart 1

tetramethyl D-fructoside and methyl 2,3,4,6-tetramethyl D-glucoside were proved by GLC. The methylation was also done with sodium hydride and methyl iodide in dimethyl sulfoxide,[6] 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.

Many fructans have been found as reserve carbohydrates in various plants and they differ from each other in structure and in molecular weight.[7] They are divided into two main groups, that is, inulin group and phlean group.[8] 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 D-fructose, 1,3,4,6-tetramethyl D-fructose and 2,3,4,6-tetramethyl D-glucoce as the products of methanolysis after methylation. The other trimethyl, dimethyl and monomethyl 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.

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. Molecular weight was determined by the use of Knauer vapor pressure osmometer. GLC was carried out by the use of Hitachi model 063 gas chromatograph equipped with hydrogen flame ion detector.

Isolation of the Fructan——The dried Ophiopogonis Tubers (10 g) were crushed and extracted with water (100 ml) for 1 hr at 40° under stirring. After suction filtration, the filtrate was concentrated and applied to a column (2×30 cm long) of active charcoal (for chromatographic use, Wako-Junyaku Co.), followed by successive elution with water (480 ml), 5% ethanol (520 ml), 10% ethanol (760 ml), 15% ethanol (800 ml), 20% ethanol (920 ml), 25% ethanol (600 ml), and 30% ethanol (560 ml). Each fraction was concentrated and lyophilized. The yields of the eluates from the extract were 9.9% in water, 2.4% in 5% ethanol, 5.2% in 10% ethanol, 10.9% in 15% ethanol, 21.0% in 20% ethanol, 6.4% in 25% ethanol and 0.3% in 30% ethanol. The eluate with 25% ethanol (1.5 g) was dissolved in water and applied to a column (3×106 cm) of Sephadex G-25 (Pharmacia Co., fine) and fractions collected at 10 ml. The carbohydrates in eluates were measured by phenol-sulfuric acid method.[9] The eluates obtained from tubes 35 to 40 were combined and concentrated, then lyophilized. Yield, 0.98 g. Further purification of this fraction with Sephadex G-25 was carried out in the same way, and the eluates obtained from tubes 36 to 38 were combined and lyophilized after concentration. Yield, 0.31 g. Chromatograms on Sephadex G-25 are shown in Fig. 1.

Analysis by TLC——TLC using Avicel SF cellulose was carried out in the usual way.[10] For checking on the purity of the fructan, solvent A, BuOH: pyridine: H_2O (1: 1: 1), was used, and the development followed by dryness in air was repeated three times. *Rf* value of the fructan was 0.13. For the purpose of analysis of the hydrolysate, the following solvent systems were used: B, BuOH: pyridine: AcOH: H_2O

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(10:6:1:3); C, BuOH: AcOH: EtOH: H_2O (3:2:1:1). The condition of the hydrolysis and *Rf* values of the hydrolysate were similar to those described in the former report of this series.[1] Sugars were revealed with naphthoresorcinol-phosphoric acid reagent[11] and benzidine reagent.[12]

GLC of Methanolysate——The sample was methanolyzed with 3% methanolic HCl at 60° for 2 hr. After cooling, the solution was treated with Amberlite IR4B (OH^-) to remove HCl, then evaporated to dryness. The residue was trimethylsilylated by the method of Sweeley, *et al.*,[13] then applied to a gas chromatograph using a column (0.3 cm × 1 m long stainless steel) packed with 3% SE 52 on Chromosorb W (80 to 100 mesh) at 140° with a flow of 20 ml per min of nitrogen; t_R, trimethylsilylated methyl D-fructoside 8.6, 9.7; trimethylsilylated methyl D-glucoside 13.1, 14.5.

Colorimetric Determination of Component Sugars——Total carbohydrates was determined by carbazole method,[14] and fructose was estimated by resorcinol method.[15] From these results, the amount of glucose could be calculated. The results revealed that the sample contains 94.9% of fructose and 5.1% of glucose (Percentages are for anhydrosugars).

Periodate Oxidation and Smith Degradation——The sample (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.[16] The oxidation was completed after two days, then the half of the solution was used for the measurement of formic acid liberation by a titration with 0.01N NaOH after addition of ethyleneglycol (0.02 ml). The residuary half of the reaction mixture was reduced with sodium borohydride (50 mg) at 5° overnight, then acetic acid was added up to pH 5. The solution was passed through a column (2 × 9 cm) of Dowex 50W-X8 (H^+) and a column (2 × 5 cm) of Dowex 44 (OH^-) successively. The eluate and the washing were combined and evaporated to dryness. The residue (3 mg) was hydrolyzed with 0.2N HCl (0.3 ml) at 100° for 6 hr or with 0.5N HCl at 60° for 2 hr. After removal of HCl by evaporation *in vacuo*, the hydrolysate was dissolved in pyridine (0.2 ml) containing trimethylolpropane (0.5 mg) as an internal standard, then subjected to trimethylsilylation by addition of hexamethyldisilazane (0.06 ml) and trimethylchlorosilane (0.03 ml). The product was applied to a gas chromatograph.

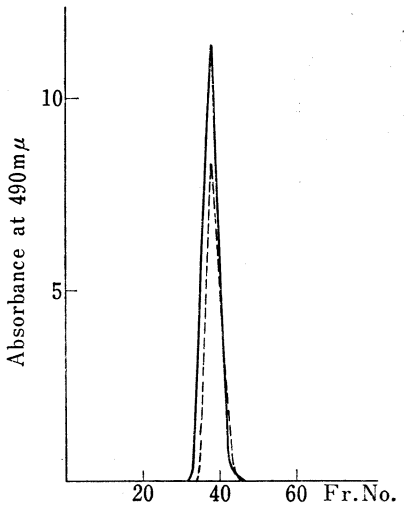

Fig. 1. Chromatogram on Sephadex G-25

———: the first eluate
- - - - - - -: the second eluate

GLC: column, 5% SE 30 on Chromosorb G (80 to 100 mesh) (0.3 cm × 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); t_R, glycerol 19.5; trimethylolpropane 23.0.

Methylation——The asmple (40 mg) was dissolved in the mixture of dimethylformamide (1 ml) and dimethyl sulfoxide (1 ml), then BaO (0.2 g), $Ba(OH)_2$–$8H_2O$ (0.2 g) and dimethyl sulfate (0.7 ml) were added successively under stirring and ice cooling. The reaction mixture was stirred at 20° for 48 hr. After addition of chloroform (12 ml), the mixture was centrifuged and the upper layer was washed with 2N NH_4OH (4 ml) followed by washing thrice with water (4 ml) containing one drop of 0.2N AcOH. The solution was dried with Na_2SO_4, filtered and evaporated *in vacuo*.

On the other hand, sodium hydride (40 mg) was mixed with dimethyl sulfoxide (5 ml) and the mixture was stirred at 70° for 1 hr. The sample (20 mg) was dissolved in dimethyl sulfoxide (3 ml) and the solution was added into this mixture. After 5 hr stirring at room temperature, methyl iodide (2 ml) was added and the reaction mixture was stirred overnight at room temperature. All procedures were carried out in nitrogen atmosphere. After dilution with water (50 ml), the mixture was extracted with chloroform (50 ml) four times. The extract was dried with Na_2SO_4 and the filtrate was evaporated *in vacuo*.

The methylation under each condition was performed again similarly. The infrared spectra of the final products had no absorption near 3400 cm^{-1}.

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Analysis of Methylation Product——The product (20 mg) was dissolved in the mixture of 3 ml of methanol and 1 ml of water containing oxalic acid (40 mg). The solution was heated in a sealed tube at 75° for 20 hr, then neutralized with $CaCO_3$ and filtered. The filtrate was evaporated to dryness, then dissolved in 0.5% methanolic HCl (2 ml) and left at room temperature for 16 hr. The solution was treated with Amberlite IR4B (OH^-) to remove HCl, then evaporated to dryness. This methanolysate was used for the identification of methyl ethers of D-fructose. For the detection of methyl ether of D-glucose, further treatment with 4% methanolic HCl was carried out in a sealed tube at 75° for 16 hr, followed by removal of HCl. Chloroform solution of the methanolysate was applied to a gas chromatograph. Following two conditions were used; A, a column (0.3 cm × 2 m long stainless steel) packed with 15% Poly-butane 1,4-diol succinate on Chromosorb W (80 to 100 mesh) at 175° with a flow of 20 ml per min of N_2; B, a column (0.3 cm × 2 m long stainless steel) packed with 5% Neopentylglycol succinate on Chromosorb G (60 to 80 mesh) at 150° with a flow of 20 ml per min of N_2. Table I shows relative retention times of the products obtained by methanolysis to methyl 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside in the two conditions.

TABLE I. Relative Retention Times of Methylation Products

	Condition A (15% BDS)	Condition B (5% NPGS)
Methyl 2,3,4,6-tetra-O-methyl D-glucoside	1.00, 1.36	1.00, 1.43
Methyl 1,3,4,6-tetra-O-methyl D-fructoside	1.05, 1.24	1.07, 1.28
Methyl 3,4,6-tri-O-methyl D-fructoside	2.44, 3.55	2.47, 3.67

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Studies on the Syntheses of Heterocyclic Compounds. DX.[1] A Novel Rearrangement of proerythrinadienol with Methyl Fluorosulfonate

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We have previously reported that phenol oxidation and irradiation of the phenolic 1-benzylisoquinolines gave proerythrinadienones, (I)[3a] and (II),[3b] respectively. This type of compound (I) is proved to be the precursors in biosynthesis of the erythrina[4] (III) and the aporphine alkaloids (IV).[5] Battersby also suggested that the dienone like I would be the precursor to hasubanan type alkaloids (VII).[6] Furthermore, dienone-phenol rearrangement of dienone (II) was investigated under various kinds of conditions, but failed.[3b] Therefore, rearrangement of this dienone (II) to thalicsimidine-type aporphine (V) was examined

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