Chem. Pharm. Bull. 16(1) 113—116 (1968)

UDC 547.458.05 : 581.19

Water-soluble Carbohydrates of Ophiopogonis Tuber. II.[1] Purification, Properties and Structures of Three Oligosaccharides

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(Received April 1, 1967)

The main oligosaccharide fraction obtained from the water extract of the tuberous roots of *Ophiopogon japonicus* Ker-Gawler var. *genuinus* Maximowicz was purified by the gel filtration on Sephadex G-25, and separated into three oligosaccharides. They were a heptasaccharide composed of one glucose unit and six fructose units, a hexasaccharide composed of one glucose unit and five fructose units, and a pentasaccharide composed of one glucose unit and four fructose units.

Methylation and periodate oxidation studies showed that the each oilgosaccharide possesses the structure consisted of a chain of 2→1 linked D-fructofuranose units having a D-glucopyranose residue, joined by a type of sucrose bond, on the end.

In the previous paper,[1] the presences of D-glucose, D-fructose, sucrose and the other oligosaccarides composed of D-glucose and D-fructose in the water extract obtained from the tuberous roots of *Ophiopogon japonicus* Ker-Gawler var. *genuinus* Maximowicz were described. As the result of fractionation by the use of chromatography on a column of charcoal-Celite, it had been shown that the yield of the total oligosaccharides except sucrose was more than a half of the amount of the dried material, but the each oligosaccharide fraction had not been purified into a pure substance yet.

The main oligosaccharide fraction has now been treated repeatedly on a Sephadex column and three pure oligosaccharides were obtained, and their properties and structures are described in the present paper. The water extract of the material was absorbed on a charcoal-Celite column, and seven fractions were eluted with water and further stepwise increments of ethanol as described in the previous report. The main oligosaccharide fraction was obtained from the eluate with 20% ethanol, after concentration and dryness *in vacuo*. The gel filtration

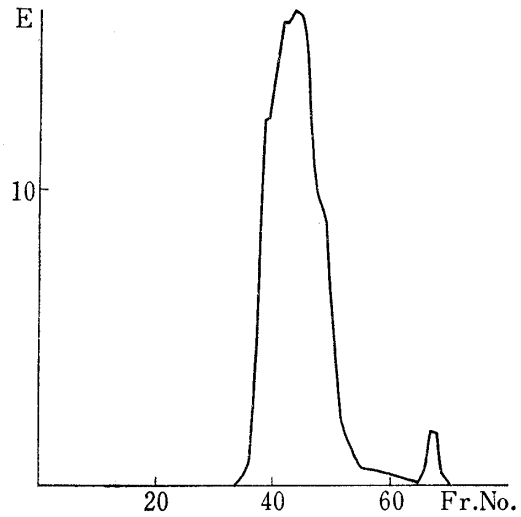

Fig. 1. Gel Filtration on Sephadex

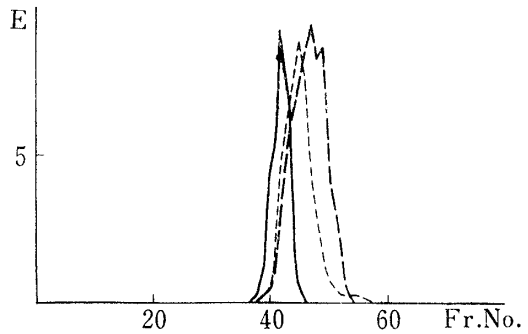

Fig. 2. Re-separation on Sephadex
——— "a", ... "b", – – – "c"

1) Part I: M. Tomoda and S. Katô, *Shôyakugaku Zasshi*, **20**, 12 (1966).
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of the water solution of the fraction was repeatedly carried out on a Sephadex G–25 column, and the three oligosaccharides which are homogeneous on cellulose thin-layer chromatography separately were obtained (Fig. 1 and 2). The authors named provisionally them oligosaccharide "A", which is a heptasaccharide, oligosaccharide "B", which is a hexasaccharide, and oligosaccharide "C", which is a pentasaccharide, as being shown respectively in the later part of this paper.

It was appeared that the component sugars of the each oligosaccharide are D–glucose and D–fructose with thin–layer chromatography of the hydrolysate. They were obtained as white powders and easily soluble in water. Specific rotations of them were as follows: "A", $[\alpha]_D^{20}=-33°$ ($c=2$, H_2O), "B", $[\alpha]_D^{20}=-32°$ ($c=2$, H_2O), and 'C'", $[\alpha]_D^{20}=-28°$ ($c=2$, H_2O). The molecular weights of them gave values of 1150 in "A", 1000 in "B", and 850 in "C" by a modification of Barger's method.[3] The results of quantitative determinations of sugar components of the oligosaccharides are given in Table I.

Table I. Sugar Composition (%) of Oligosaccharides

	Fructose	Glucose
Oligosaccharide "A"	84.7	14.6
Oligosaccharide "B"	84.0	17.0
Oligosaccharide "C"	80.5	20.1

Owing to these results, it is clear that "A" is a heptasaccharide composed of one glucose unit and six fructose units, "B" is a hexasaccharide composed of one glucose unit and five fructose units, and "C" is a pentasaccharide composed of one glucose unit and four fructose units.

As the result of periodate oxidation, 7.81 mole of periodate per one mole of "A" was consumed with 1.10 mole of formic acid liberation. In case of "B", 7.08 mole of periodate per one mole of the substrate was consumed with 0.89 mole of formic acid liberation, and 5.90 mole of periodate per one mole of the substrate was con-

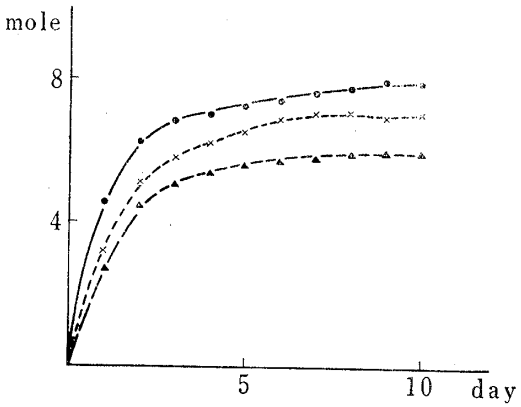

Fig. 3. Periodate Oxidation
——— "A", ... "B", – – – "C"

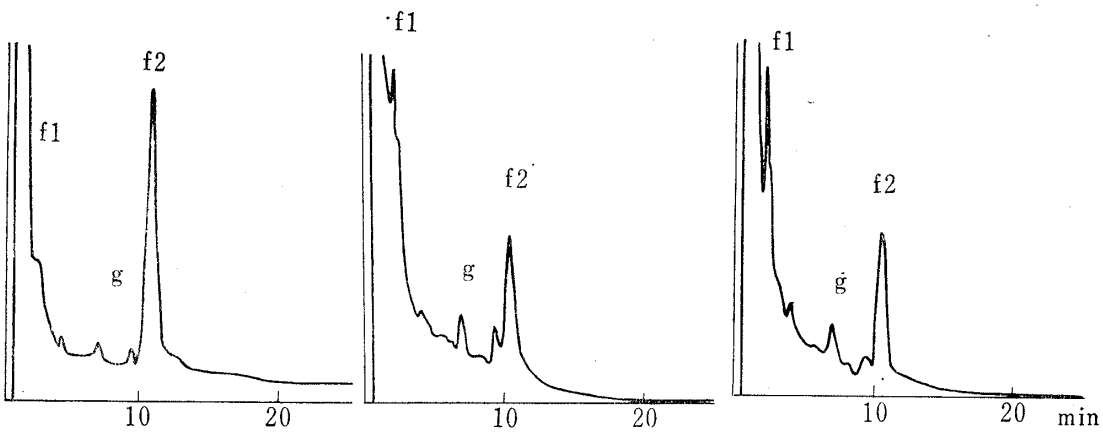

Fig. 4. Gas Chromatogram of Methanolyzate of Methylated "A" (1), Methylated "B" (2), and Methylated "C" (3)
f1 : 1,3,4,6–Me–Fru, f2 : 3,4,6–Me–Fru, g : 2,3,4,6–Me–Glc

3) S. Akiya, *Yakugaku Zasshi*, **57**, 967 (1937).

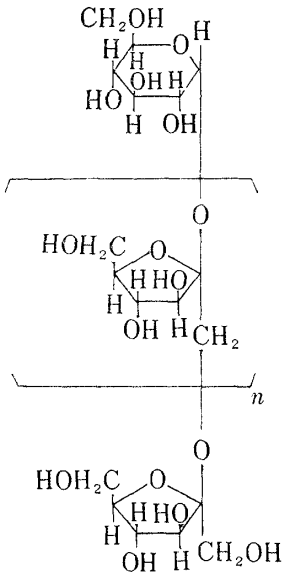

Chart 1

"A": $n=5$
"B": $n=4$
"C": $n=3$

sumed with 0.91 mole of formic acid liberation in case of "C" (Fig. 3).

After methylation with sodium hydride and methyl iodide in dimethyl sulfoxide,[4] methylated products were methanolyzed. Gas liquid chromatography of the methanolyzate of each oligosaccharide showed the presence of methyl 3,4,6-trimethyl D-fructoside, methyl 1,3,4,6-tetramethyl D-fructoside and methyl 2,3,4,6-tetramethyl D-glucoside (Fig. 4).

From these results, the structure illustrated in Chart 1 could be proposed to the three oligosaccharides obtained from Ophiopogonis Tuber. It is well known that D-fructofuranose polymers occur as reserve substances in various plants, particularly those in the Compositae and Gramineae families. And the presence of the less polymerized members of a series of glucofructans having the structure with 2→1 linkage in the tubers of *Helianthus tuberosus* L. has been reported by several authors.[5-7] The oligosaccharides described in the present paper are new members of this series, and it is interesting that there are such substances in rich in the extract of the tuberous root of a plant in the Liliaceae family.

Experimental

Isolation of Main Oligosaccharide Fraction——This was similar to that described in the previous report. The dried Ophiopogonis Tubers (10 g) were crushed, then extracted with water (100 ml) for 1 hr in hot water bath. After suction filtration, the filtrate was concentrated *in vacuo* and applied to the top of a charcoal Celite 535 (1:1) column (4×22 cm), followed by successive elution with water (0.8 liter), 5% ethanol (1 liter), 10% ethanol (0.8 liter), 15% ethanol (1.3 liter), 20% ethanol (1.8 liter), 25% ethanol (1.3 liter) and 30% ethanol (1 liter). The fractions eluted with 20% ethanol were collected and concentrated *in vacuo* to dryness. Yield, 26.8%.

Gel Filtration on a Sephadex Column——Sephadex G-25 (Pharmacia Co.) was stirred and decantated to remove fines and washed repeatedly with distilled water. For the separation of 1.4 g of the sample, a Sephadex G-25 column (3×105 cm) was used and fractions were collected at 10 ml for 20 min. The oligosaccharides in eluates were measured by phenol-sulfuric acid method[8] (E at 490 mμ). The eluates obtained from the column were divided into three groups: Frac. 1, tubes 37 to 40; Frac. 2, tubes 41 to 42; Frac. 3, tubes 43 to 44 in Fig. 1. The yields were 0.24 g in Frac. 1, 0.23 g in Frac. 2 and 0.63 g in Frac. 3. The each fraction was concentrated *in vacuo*, then re-separation with Sephadex G-25 was carried out separately. The results of gel filtrations of Frac. 1, 2 and 3 are respectively shown in "a", "b" and "c" of Fig. 2. The oligosaccharide "A" (0.18 g) was obtained from tubes 43 to 44 in "a" and "b". The oligosaccharide "B" (0.3 g) was obtained from tubes 47 to 48 in "b" and from tubes 41 to 46 in "c". And the oligosaccharide "C" (0.16 g) was obtained from tubes 49 to 52 in "c".

Thin-layer Chromatography——Oligosaccharides were hydrolyzed with 0.5 N-sulfuric acid at 60° for 2 hr, then neutralized with barium carbonate. The procedure of thin-layer chromatography was similar to that described in the other papers,[9] but the following solvent systems were used: A, BuOH: pyridine: HOAc:H_2O (10:6:1:3, by vol.); B, BuOAc:HOAc:EtOH:H_2O (3:2:1:1, by vol.); C, BuOH:pyridine:H_2O (1:1:1, by vol.). The former two were used for the separation of component monosaccharides and the last

4) S. Hakomori, *J. Biochem.*, **55**, 205 (1964).
5) R. Dedonder, *Bull. Soc. Chim. Biol.*, **34**, 144 (1952).
6) I.R. Rominskii, A.S. Sushkova, and A.V. Ilina, *Ukrain. Khim. Zhur.*, **24**, 236 (1958); (*C.A.*, **52**, 15663*b* (1958)).
7) T.G. Jefford and J. Edelman, *J. Exptl. Botany*, **12**, 177 (1961); *ibid.*, **14**, 56 (1963); (*C.A.*, **56**, 6374*d* (1962); *C.A.*, **60**, 14837*f* (1964)).
8) M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, *Anal. Chem.*, **28**, 350 (1956).
9) M. Tomoda: *Yakugaku Zasshi*, **87**, 207 (1967); M. Tomoda and M. Kitamura, *Chem. Pharm. Bull.* (Tokyo), in press.

was used for checking on the purities of oligosaccharides. The sugars spots were revealed with benzidine reagent.[10] Table II shows *Rf* values of sugars in the three solvent systems.

Table II. Rf Values of Sugar Components and Oligosaccharides

	Solvent A	Solvent B	Solvent C
D-Fructose	0.40	0.39	
D-Glucose	0.34	0.30	
Oligosaccharide "A"			0.11
Oligosaccharide "B"			0.21
Oligosaccharide "C"			0.34

Determination of Sugars——Total carbohydrate was determined by carbazole method,[11] and fructose was estimated by resorcinol method.[12] From these results, the amount of glucose could be computed.

Periodate Oxidation——The sample (10 mg) was oxidized with 0.1 M sodium metaperiodate (2 ml) at 5° in a dark place. The periodate consumption was measured by a spectrophotometric method.[13] The oxidation was completed after 10 days, then the formic acid liberation was measured by a titration method.[14]

Methylation——The oligosaccharide (10 mg) was dissolved in dimethyl sulfoxide (10 ml) and treated with sodium hydride (100 mg) in dimethyl sulfoxide (5 ml) a little at a time under stirring. After 30 minutes, methyl iodide (5 ml) was added carefully and the mixture was stirred overnight in a glass-stoppered flask at room temperature. The reaction mixture was diluted with water (8 ml) and was followed by extraction of the methylated products with chloroform (5 ml) thrice. The extracted solution was then evaporated *in vacuo*. The methylation was repeated twice, and the infrared spectra of the product had no absorption near 3400 cm^{-1}.

Gas-liquid Chromatography of Methanolyzate——The fully methylated product described above was heated with 3% methanolic HCl (4 ml) in a sealed tube at 100° for 5 hr. After cooling, the solution was treated with Amberlite IR4B (OH^- form) to remove HCl, then evaporated *in vacuo*. The chloroform solution of the residue was applied to gas-liquid chromatography using a Hitachi model F6D with 5% Neopentylglycol succinate on Chromosorb G (1 m) at 180° and with a flow of 20 ml/minute of nitrogen.

10) J.S.D. Bacon and J. Edelman, *Biochem. J.*, **48**, 114 (1951).
11) F.B. Seibert and J. Atno, *J. Biol. Chem.*, **163**, 511 (1946).
12) R.G. Kurka, *Biochem. J.*, **63**, 542 (1956).
13) J.S. Dixon and D. Lipkin, *Anal. Chem.*, **26**, 1092 (1954).
14) D.M.W. Anderson, C.T. Greenwood, and E.L. Hirst, *J. Chem. Soc.*, **1955**, 225.