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Two Oligosaccharides Formed by Onion Sucrose: sucrose 1^F-β-D-fructosyltransferase from Onion Seeds

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Introduction

We have described the purification and properties of fructosyltransferases from liliaceous plants[16~19,22]. We previously studied substrate specificities of sucrose: sucrose 1^F-β-D-fructosyltransferase (EC 2, 4, 1, 99; SST) from onion seeds[22] and asparagus roots[19]. We showed onion SST catalyzed the fructosyl transfer from donor sucrose to fructosyl residue of acceptor saccharides, raffinose and stachyose other than sucrose to produce a tetra- and a penta-saccharide. However, the structure of the saccharides produced by the SST action has not been investigated. We now report the isolation and structural determination of two oligosaccharides formed by the action of SST from onion seeds.

Materials and Methods

Materials. Onion seeds (*Allium cepa* L. var. Early yellow globe) were purchased from commercial sources (Sapporo Konoen Ltd.). Sucrose, raffinose and stachyose were obtained from Wako Junyaku Co. Ltd.. 1-Kestose [*O*-β-D-Fruf-(2→1)-*O*-β-D-Fruf-(2→1)-α-D-Glcp], and 1^F, 6^G-di-β-D-fructofuransylsucrose {*O*-β-D-Fruf-(2→1)-*O*-[β-D-Fruf-(2→6)-*O*-α-D-Glcp-(1→2)]-β-D-Fruf} were isolated from asparagus roots as previously described[20].

Onion sucrose: sucrose 1^F-β-D-fructosyltransferase (97 U/mg of protein) were purified according to the method reported in the previous paper[22]. One unit of the enzyme activity was defined as the amount of enzyme transferring a D-fructose group from sucrose to sucrose that produces 1 μmole of 1-kestose in 1 h at 0.4 M of sucrose in McIlvaine buffer (pH 5.4) and 30°C.

Quantitative determination of sugars. Total hexoses, ketoses and reducing sugars were quantitatively determined by anthrone[10], Roe's[15], and Somogyi-Nelson's[12,23,24] methods, respectively. Glucose was determined with commercial Glucostat reagent.

Paper chromatography (PC) and thin-layer chromatography (TLC). Two solvents, 1-propanol-ethyl acetate-water (7 : 1 : 2) (I) and 1-propanol-ethyl acetate-water (6 : 1 : 3) (II), were used for PC (Toyo No. 50 filter paper), and a solvent, 1-butanol-2-propanol-water (10 : 5 : 4) (III), was employed for TLC [Kieselgel G (Typ 60) containing gypsum by 13% and pre-coated TLC plates, Kieselgel 60 F-254, E. Merck, Darmstadt]. After double to quintuple development, sugars were detected

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by anisidine phosphate[11] and alkaline silver nitrate reagents[27].

Isolation of oligosaccharides. A mixture (a) of onion SST (25 U), 0.2 M sucrose and 0.1 M raffinose in McIlvaine buffer (0.2 M sodium hydrogen phosphate — 0.1 M citric acid system : pH 5.4, 20 ml) was incubated at 30°C for 24 h. Another mixture (b) of onion SST (25 U), 0.2 M sucrose and 0.1 M stachyose in the same buffer (10 ml) was incubated under the same condition. After the reaction was halted by heating at 100°C, each aliquot of mixture (a) or (b) was chromatographed on paper with solvent I. A tetrasaccharide corresponding to fructosyl raffinose from sucrose and raffinose or a pentasaccharide corresponding to fructosyl stachyose from sucrose and stachyose was produced by onion SST. Also, formation of each saccharide was accompanied with that of 1-kestose from sucrose.

Reaction mixture (a) was applied on a charcoal-Celite column (5.5×45 cm) and eluted with 10% ethanol (3 l) and 30% ethanol (2.5 l). The yields of the eluates as dry matter were as follows; 10% ethanol fraction, 1.517 g, and 30% ethanol fraction, 0.280 g. Thirty percent ethanol fraction, estimated to contain the bulk of tetrasaccharide was subjected to preparative paper chromatography with a solvent mixture II, and a band corresponding to that of the tetrasaccharide was excised from paper and extracted with water. The extract was desalted with Amberlite IR-120 B (H^+) and IRA-410 (OH^-), decolorized with a minimum of charcoal, concentrated *in vacuo* and lyophilized to give a white powder (saccharide A, 0.162 g).

Reaction mixture (b) was chromatographed on a charcoal-Celite column (4.5× 50 cm) with 10% ethanol (2 l) and 30% ethanol (3 l). The yields of the eluates as dry matter were 730 mg (10% ethanol fraction) and 325 mg (30% ethanol fraction).

Thirty percent ethanol fraction, containing a pentasaccharide was purified in a similar manner as described above to give a white powder (saccharide B, 58 mg).

Methylation and methanolysis. Methylation of the isolated saccharides was conducted by the method of Hakomori[4] as described in the previous paper[20], and the methylated saccharides were methanolyzed by heating with 1.5% methanolic hydrochloric acid at 92°C for 30 min. The reaction mixture was treated with Amberlite IRA-410 (OH^-) to remove hydrochloric acid, and evaporated *in vacuo* to dryness. The methanolyzate was dessolved in a small quantity of methanol and subjected to gas chromatography.

Gas liquid chromatography (GLC). GLC was carried out using a Shimadzu GC8A gas chromatograph with a glass column (2.6 mm×2 m) packed with 15% butane 1, 4-diol succinate polyester on acid-washed Celite at 175°C for analysis of the methanolyzate and packed with 5% SE 52 on chromosorb WAW DMCS at 125°C～280°C (8°C/min) for that of TMS-derivatives prepared by the method of Sweeley *et al.*[25] Flow rate of nitrogen carrier gas were 40 ml/min.

Hydrolysis. (1) Partial hydrolysis : the isolated saccharide (2 mg) was dissolved in 0.05 N oxalic acid (0.5 ml) and partially hydrolyzed by heating at 60°C for 15 min ; (2) Complete hydrolysis : the isolated saccharide (2 mg) was dissolved in 0.5 N hydrochloric acid (0.5 ml) and hydrolyzed by heating at 100°C for 1 h ; (3) Enzymatic hydrolysis : the isolated saccharide (2 mg) was hydrolyzed by incubating with β-fructofuranosidase or α-galactosidase (0.2 ml ; 0.4 mg of Sigma VI yeast β-fructo-

furanosidase or of Sigma green coffee α-galactosidase in 0.2 ml of McIlvaine buffer, pH 5.5) at 30°C for 15 hr.

High performance liquid chromatography (*HPLC*). HPLC was carried out by use of a Jasco TRIROTAR-VI system with a column (8×300 mm, Shodex sz 5532, Showadenko Co. Ltd.) at 65°C. The column was eluted with acetonitrile-water (70 : 30, v/v) at 1 ml/min. Detection was done with a differential refractometer RID-300.

^{13}C-NMR spectrometry. ^{13}C-NMR spectra of the saccharides in D_2O were recorded on a JEOL NMR spectrometer (JNM-GX 270) using dioxane as an internal reference standard.

Field desorption mass spectrometry (FDMS). FDMS spectra were measured with a JEOL mass spectrometer (JMS-OISG-2).

Results and Discussion

Saccharides A ($[\alpha]_D^{25}+80.2$) and B ($[\alpha]_D^{25}+100.2$) were shown to be homogenious by PC ($R_{glucose}$: 0.35 and 0.13), TLC ($R_{glucose}$: 0.30 and 0.14, pre-coated plate) and HPLC ($t_{Rglucose}$: 2.59 and 4.46). They were non reducing, and on hydrolysis with 0.5 M hydrochloric acid or α-galactosidase and β-fructofuranosidase gave D-fructose, D-glucose and D-galactose. The saccharides released from both saccharides A and B by the action of α-galactosidase were only galactose and 1-kestose. The degrees of polymerization were established to be 4 (A) and 5 (B) by measurements of the $[M+Na]^+$ ions (m/z: A, 689 and B, 851) on FDMS, and molar ratios (A, 1.85 : 1.0 : 1.1 ; B. 1.80 : 1.0 : 2.05) of D-fructose, D-glucose and D-galactose in acid hydrolyzates of the saccharides by HPLC.

Thus, the saccharides A and B were found to be a tetrasaccharide and a pentasaccharide made up of fructose (A, B : 2 mol), glucose (A, B : 1 mol) and galactose (A : 1 mol, B : 2 mol).

The saccharides A and B were permethylated by Hakomori's method[4]. The permethylated saccharides were methanolized with 1.5% methanolic hydrogen chloride and were subjected to GLC. The GLC patterns and relative retention times (t_R) of the methanolyzates of the permethylated saccharides are shown in Fig. 1 and Table 1.

The methanolyzate of the permethylated saccharide A gave seven peaks corresponding to methyl 1, 3, 4, 6-tetra-*O*-methyl fructoside (t_R, 1.03 and 1.26), methyl 3, 4, 6-tetra-*O*-methyl fructoside (t_R, 2.68 and 3.96), methyl 2, 3, 4-tri-*O*-methyl-glucoside (t_R, 2.50 and 3.53) and methyl 2, 3, 4, 6-tetra-*O*-methyl galactoside (t_R, 1.70). The methanolyzate of the permethylated saccharide B gave one more peak corresponding to methyl 2, 3, 4,-tri-*O*-methyl galactoside (t_R, 7.06) in addition to these peaks observed in that of permethylated saccharide A.

From these findings, saccharides A and B were proved to be 1^F-β-D-fructosyl raffinose and 1^F-β-D-fructosyl stachyose.

The structural confirmation of saccharides A and B was made by ^{13}C-NMR analysis. ^{13}C-NMR data for saccharide A, saccharide B and reference saccharides ; 1-kestose[7] and raffinose[5] were shown in Fig. 2 and Table 2. The general assign-

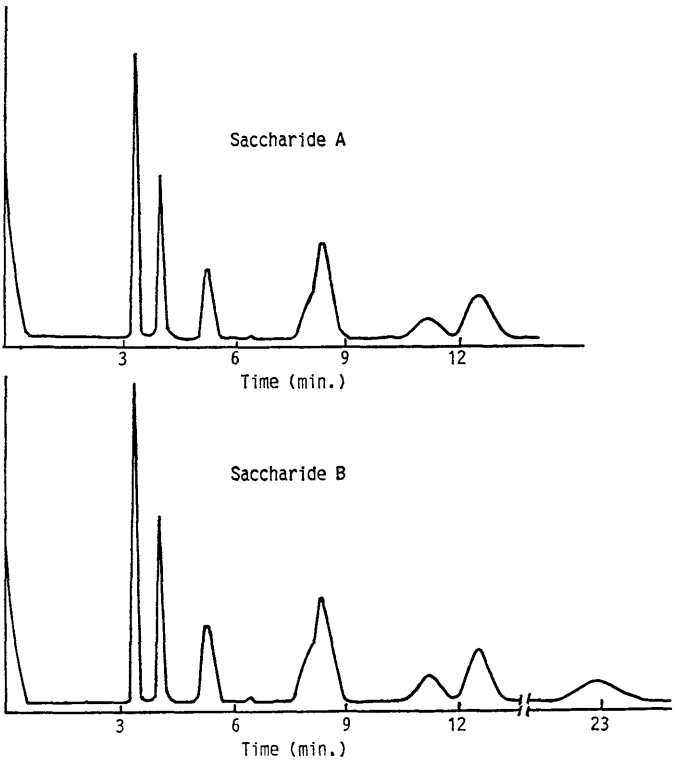


Fig. 1. Gas-liquid chromatograms of methanolyzates of permethylated oligosaccharides synthesized by onion SST.

Table 1. GLC-analysis of methanolysis products of permethylated saccharides A and B

Methanolyzate origin	Relative retention time[a]							
Saccharide A	1.03	1.26	1.70	2.50	2.68	3.53	3.96	
Saccharide B	1.03	1.26	1.70	2.51	2.69	3.54	3.98	7.06
1^F, 6^G-Di-β-D-fructofuranosyl sucrose[b]	1.03	1.26		2.50	2.68	3.54	3.96	
Raffinose[b]	1.03	1.26	1.70	2.50		3.52		
Stachyose[b]	1.03	1.26	1.70	2.51		3.54		7.06
Methyl 2, 3, 4, 6-tetra-*O*-methyl α or β-D-galactoside[c]			1.72 or 1.68					
Methyl 2, 3, 4, 6-tetra-*O*-methyl-β-D-glucoside[c]	1.00							

a) Retention time of methyl 2, 3, 4, 6-tetra-*O*-methyl-β-D-glucoside = 1.

b) Reference methanolyzate. Specimens of 1^F, 6^G-di-β-D-fructofuranosyl sucrose was previously isolated from asparagus roots[20].

c) Reference methylated sugars, which were prepared from methyl β-D-glucoside, methyl α- or β-D-galactoside.

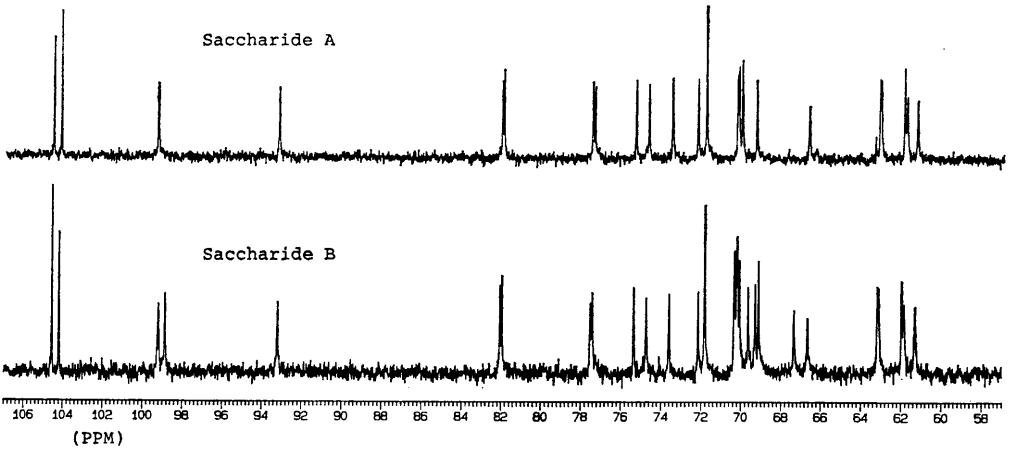


Fig. 2. ^{13}C-NMR Spectra of saccharides A and B formed by onion SST.

ment of resonances in the spectrum of saccharide A in D_2O was tentatively made by comparison of the observed chemical shifts with the data of 1-kestose and raffinose. The spectrum of difructosyl residue in saccharide A was noted to resemble to that of difructosyl residues in 1-kestose[7]. Also, the same interpretation facilitated the assignment of resonances in the spectrum of glucosyl and galactosyl residues in saccharide A by referring the carbon resonances of raffinose[1,5] although the signal assignment was not always unambiguous.

The resonance (δ 61.96) of C-1 of inner fructosyl residue in saccharide A were sensitively deshielded with respect to that of the terminal D-fructosyl residue by 0.65 ppm suggesting that the C-1 of inner D-fructosyl residue was linked glycosidically with C-2 of terminal fructose. The resonances at δ 104.56 and δ 104.20 were assigned to C-2 of the inner and terminal fructosyl residues of saccharide A, respectively by running a comparison with the spectrum data of 1-kestose[7], sucrose[1,3,6,7,9], inulin[7,26] and grass levan[7,2]. The resonance at δ 75.35 was attributed to C-4 of the terminal fructosyl residue in the sucrose group of saccharide A by comparing the chemical shifts of C-4 in methyl-β-D-fructofuranoside[2], sucrose[7] and 1-kestose[7].

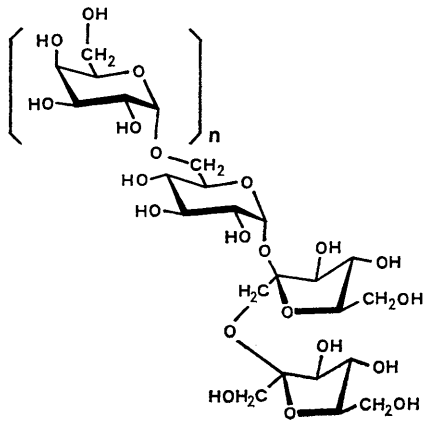
The chemical shifts of carbons of glucosyl and galactosyl residues in saccharide A were similar to those of carbons of the both residues in raffinose. Each carbon signal except for C-4 signal of glucosyl residue and C-3 signal of galactosyl residue in saccharide A were assigned as shown in Table 2.

A similar argument enables the preliminary assignment of carbon signals of saccharide B by comparison with the signal data for reference saccharides, 1-kestose[7] and stachyose[1]. Each carbon of difructosyl residue in saccharide B showed a quite similar chemical shift to that of difructosyl residue in 1-kestose and saccharide A, although the assignment of carbon signals of digalactosyl residue in saccharide B was almost equivocal. The resonances at δ 98.88 and 99.21, or δ 61.86 and 67.35 were assigned to C-1, or C-6 of the terminal and inner galactosyl residues of saccharide B by referring to the corresponding chemical shifts (δ 99.0 and 99.3, or δ 62.1 and 67.4) of stachyose reported by Allerhand[1] *et al.*

Table 2. ^{13}C-NMR chemical shifts of saccharides A and B formed by onion SST

Carbon atom	Saccharide A (Fructosyl raffinose)	Saccharide B (Fructosyl stachyose)	1-Kestose	Raffinose
Terminal fructose				
C-1	61.31	61.33	61.7	
C-2	104.20	104.20	104.5	
C-3	77.41	77.42	77.9 (76.4 in Me_2SO-d_6)	
C-4	75.35	75.35	75.7	
C-5	81.97[a]	81.96[a]	82.4[a]	
C-6	63.12[b]	63.11[b]	63.4[b]	
Inner fructose				
C-1	61.96	61.97	62.2	62.23
C-2	104.56	104.55	104.9	104.60
C-3	77.51	77.51	77.9 (76.9 in Me_2SO-d_6)	77.16
C-4	74.73	74.73	75.1	74.81
C-5	82.06[a]	82.06[a]	82.4[a]	82.15
C-6	63.18[b]	63.17[b]	63.5[b]	63.27
Glucose				
C-1	93.23	93.22	93.7	92.90
C-2	71.86	71.82	72.4	71.77
C-3	73.55	73.61	73.8	73.48
C-4	70.24[c]	70.31[c]	70.5	70.25
C-5	72.28	72.15	73.6	72.21
C-6	66.68	66.65	61.6	66.72
Inner galactose				
C-1	99.32	99.21		99.29
C-2	69.33	69.13[e]		69.30
C-3	70.29[c]	70.34[c]		70.25
C-4	70.06	69.65[d]		70.03
C-5	71.86	70.21[c]		71.83
C-6	61.84	67.35		61.94
Terminal galactose				
C-1		98.88		
C-2		69.29[e]		
C-3		70.21[c]		
C-4		70.08[d]		
C-5		71.82		
C-6		61.86		

Chemical shifts are expressed in ppm down field from the signal for TMS relative to which the 1, 4 dioxane signal appears at δ 67.40. The assignments of the resonances marked with a, b, c, d or e respectively, may be exchanged. Data for 1-kestose and raffinose were obtained from ref. 7 and 5, respectively.



Saccharide A (n=1)
Saccharide B (n=2)

All of ^{13}C-NMR data were in accord with the clarified structures of saccharides A and B.

From the findings described above, saccharides A and B formed by fructosyl transfer to raffinose and stachyose from sucrose with onion SST were confirmed to be 1^F-β-D-fructofuranosyl raffinose, *O*-β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl-*O* [-α-D-galactopyranosyl-(1→6)]-α-D-glucopyranoside and a new saccharide, 1^F-β-D-fructofuranosyl stachyose, *O*-β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl-{[*O*-α-D-galactopyranosyl-(1→6)]$_2$}-α-D-glucopyranoside.

Previously we reported onion[22] and asparagus[19] SST catalyzed the fructosyl transfer from sucrose to sucrose producing 1-kestose and glucose, and also transferred some fructosyl residues from sucrose to neokestose[19], raffinose[22] and stachyose[22]. In this study, we first elucidated the structures of two oligosaccharides synthesized *in vitro* by onion SST although the same saccharides whose structures had not been clarified were produced by the transfer action of *Aspergillus oryzae* invertase[13,14]. From this structural examination, onion SST was confirmed to catalyze the fructosyl transfer to the 1-hydroxyl of the fructosyl residue of the sucrose moiety in raffinose and stachyose from sucrose.

Kato *et al.*[8] reported the isolation and identification of 1^F-β-D-fructosyl raffinose and its related saccharide in cottonseeds but their synthetic pathways were not studied.

Recently, we preliminary found[21] that the onion seeds included fructosyl raffinose and fructosyl stachyose as well as 1-kestose nystose, raffinose and stachyose. These fructosyl oligosaccharides may be synthesized *in vivo* by fructosyl transfer to fructosyl residue of sucrose group in oligosaccharide with SST action.

Summary

A tetrasaccharide and a new pentasaccharide synthesized by D-fructosyl transfe. from sucrose to raffinose and stachyose with a purified sucrose : surcose 1^F-β-D-

fructosyltransferase [EC 2, 4, 1, 99] of onion seeds, were isolated and identified as 1^F-β-D-fructofuranosyl raffinose, O-β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl-O[-α-D-galactopyranosyl-(1→6)]-α-D-glucopyranoside and 1^F-β-D-fructofuranosyl stachyose, O-β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl-{[O-α-D-galactopyranosyl-(1→6)]$_2$}-α-D-glucopyranoside respectively by examination of the constituent saccharides, gas chromatographic analysis of methyl derivatives, investigation of enzyme (β-fructofuranosidase or α-galactosidase)-catalyzed hydrolysis products, and ^{13}C-NMR measurements of the isolated saccharides.

References

1) Allerhand, A. and J. Doddrell, 1971. Strategies in the Application of Partially Relaxed Fourier Transform Nuclear Magnetic Resonance Spectroscopy in Assignments of Carbon-13 Resonances of Complex Molecules. Stachyose. *J. Amer. Chem. Soc.*, **93**: 2777–2779.

2) Angyal, S. J. and G. S. Bethell, 1976. Conformational Analysis in Carbohydrate Chemistry. III The ^{13}C N. M. R. Spectra of the Hexuloses. *Aust. J. Chem.*, **29**: 1249–1265.

3) Dorman, D. E. and J. D. Robers, 1971. Nuclear Magnetic Resonance Spectroscopy. Carbon-13 Spectra of Some Common Oligosaccharides. *J. Amer. Chem. Soc.*, **93**: 4463–4472.

4) Hakomori, S., 1964. A Rapid Permethylation of Glycolipid, and Polysaccharide Catalyzed by Methylsulfinyl Carbanion in Dimethyl Sulfoxide. *J. Biochem.*, **55**: 205–208.

5) Hall, L. D. and G. A. Morris, 1980. Measurement of Carbon-13-proton Coupling-constants in Oligosacchardes by Two-dimensional Carbon-13 N. M. R. Spectroscopy. *Carbohydr. Res.*, **82**: 175–184.

6) Hough, L. and E. O'Brien, 1981. β-D-Fructofuranosyl α-D-arabino-hexopyranosid-2-ulose (2-ketosucrose). *Carbohydr. Res.*, **92**: 314–317.

7) Jarrell, H. C., T. F. Conway, P. Moyna and I. C. P. Smith, 1979. Manifestation of Anomeric form, Ring Structure, and Linkage in the ^{13}C-N. M. R. Spectra of Oligomers and Polymers Containing D-Fructose: Maltulose, Isomaltulose, Sucrose, Leucrose, 1-Kestose, Nystose, Inulin, and Grass Levan. *Carbohydr. Res.*, **76**: 45–57.

8) Kato, K., M. Abe, K. Ishiguro and Y. Ueno, 1979. Isolation and Characterization of New Tetrasaccharides from Cottonseed. *Agric. Biol. Chem*, **43**: 293–297.

9) Khan, R. and H. Lindseth, 1979. Selective De-acetylation of 1′, 2: 4, 6-Di-O-isopropylidene-sucrose Tetra-acetate. *Carbohydr. Res.*, **71**: 327–330.

10) Morris, D. L., 1948. Quantitative Determination of Carbohydrates with Dreywood's Anthrone Reagent. *Science.*, **107**: 254–255.

11) Mukherjee, S. and H. C. Srivastava, 1952. Improved Spray Reagent for the Detection of Sugar. *Nature.*, **169**: 330.

12) Nelson, N., 1944. A Photometric Adaptation of the Somogyi Method for the Determination of Glucose. *J. Biol. Chem.*, **153**: 375–380.

13) Pazur, J. H., 1952. Transfructosidation Reactions of an Enzyme of *Aspergillus oryzae*. *J. Biol. Chem*, **199**: 217–225.

14) Pazur, J. H., 1953. Substrate ad Cosubstrate Requirements for Enzymatic Transfructosidation. *J. Amer. Chem. Soc.*, **75**: 6323–6324.

15) Roe, J. H., J. H. Epstein and N. P. Goldstein, 1949. A Photometoric Method for the Determination of Inulin Plasma and Urine. *J. Biol. Chem.*, **178**: 839–845.

16) Shiomi, N., 1981. Purification and Characterisation of 6^G-Fructosyltransferase from the Roots of Asparagus (*Asparagus officinalis* L.). *Carbohydr. Res.*, **96**: 281–292.

17) Shiomi, N., 1982. Purification and Characterisation of 1^F-Fructosyltransferase from the

Roots of Asparagus (*Asparagus officinalis* L.). *Carbohydr. Res.*, **99**: 157–169.
18) Shiomi, N., 1982. Reverse Reactions of Fructosyl Transfer Catalysed by Asparagus 6^G-Fructosyltranseferase. *Carbohydr. Res.*, **106**: 166–169.
19) Shiomi, N. and M. Izawa, 1980. Purification and Characterization of Sucrose: sucrose 1-fructosyltransferase from the Roots of Asparagus (*Asparagus officinalis* L.). *Agric. Biol. Chem.*, **44**: 603–614.
20) Shiomi, N., J. Yamada and M. Izawa, 1976. Isolation and Identification of Fructo-oligosaccharides in Roots of Asparagus (*Asparagus officinalis* L.). *Agric. Biol. Chem.*, **40**: 567–575.
21) Shiomi, N., H. Kido and S. Kiriyama, 1982. Abstracts of Papers, the Annual Meeting of Hokkaido Branch of Agricultural Chemical Society of Japan, Obihiro, July, p. 15.
22) Shiomi, N., H. Kido and S. Kiriyama, 1985. Purification and Properties of Sucrose: sucrose 1^F-β-D-fructosyltransferase in Onion Seeds. *Phytochemistry*, **24**: 695–698.
23) Somogyi, M., 1945. Determination of Blood Sugar. *J. Biol. Chem.*, **160**: 69–73.
24) Somogyi, M., 1952. Note on Sugar Determination. *J. Biol. Chem.*, **195**: 19–23.
25) Sweeley, C. C., R. Bentley, M. Makita and W. W. Wells, 1963. Gas-liquid Chromatography of Trimethylsilyl Derivatives of Sugars and Related Substances. *J. Amer. Chem. Soc.*, **85**: 2497–2507.
26) Tomašić, J., H. J. Jennings and C. P. J. Glaudemans, 1978. Evidence for A Single Type of Linkage in A Fructofuranan from *Lolium perenne*. *Carbohydr. Res.*, **62**: 127–133.
27) Trevelyan, W. E., D. P. Procter and J. S. Harrison, 1950. Detection of Sugars on Paper Chromatograms. *Nature*, **166**: 444–445.

要　　約

玉葱種子から精製した Sucrose: sucrose 1^F-β-D-fructosyltransferase を用い，ショ糖からラフィノースおよびスタキオースへのフラクトース転移により各々一種類の四糖類および新しい五糖類を合成し，各種クロマトグラフィーを行うことにより両糖を単離した。これらの糖の構成糖分析，メチル化糖のガスクロマトグラフィー分析，β-フラクトフラノシダーゼおよびα-ガラクトシダーゼによる加水分解生成物の調査，および両単離糖の ^{13}C-NMR 測定などにより，これらの糖は 1^F-β-D-fructofuranosyl raffinose, *O*-β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl-*O*-[α-D-galactopyranosyl-(1→6)]-α-D-glucopyranoside および 1^F-β-D-fructofuranosyl stachyose, *O*-β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl-{[*O*-α-D-galactopyranosyl-(1→6)]$_2$}-α-D-glucopyranoside であることが確かめられた。