Structure of Fructan Prepared from Onion Bulbs (Allium cepa L.)

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Abstract: Fructan was prepared from an extract of onion bulbs by preparative paper and gel permeation chromatographies. Fructan was a mixture of saccharides with degree of polymerization (DP) and MW range of approximately 7−13 and 1,152−2,124, respectively, as determined by matrix-assisted laser desorption ionization/time of flight mass spectrometry. It yielded fructose and glucose upon hydrolysis with acid or yeast β-fructofuranosidase. The ratio of D-fructose to D-glucose in the enzyme hydrolysate was 8.7:1, determined by high performance anion-exchange chromatography analysis. Structural determinations were made on 13C-nuclear magnetic resonance spectroscopy. Numerous signals corresponding to carbon-1 (C-1), C-2, C-3, C-4, C-5, and C-6 of the D-fructose residues of fructan were observed at δ 61.04−61.81, 71.75−73.10, 74.50−75.15, 81.77, and 62.85−62.96, respectively. These chemical shifts were similar to those of inulin. Moreover, weaker signals were detected at δ 92.90, 103.70, 69.76 and 72.22 due to C-1, C-2, C-3, C-4, and C-5, respectively of the D-glucose residue. The chemical shifts are almost identical to those of the D-glucose carbons of neokestose, 6(1-β-D-fructofuranosyl)sucrose, and 1, 6'-di-β-D-fructofuranosyl sucrose. These findings were supported by analysis of the methanolyse from permethylated fructan using gas-liquid chromatography. The fructan from onion bulbs was composed of saccharides possessing approximately 6−12 D-fructose residues, linked by β-2,1 bonds, and a non-terminal D-glucose residue bound with D-fructose residues at the C-1 and C-6 positions.

Key words: fructan, fructosyl sucrose, onion, neokestose derivative

INTRODUCTION

The occurrence of fructan in several Allium species has been known since 1894, as reported by Archbold.1) The content, distribution, and structure of fructan in Allium species were first investigated, during the 1970s, by Bacon,2) Darbyshire,3,4) and Henry.3,4) Later, its content and distribution were studied by Jaime et al.5) and Campbell et al.6) These studies were characterized by a relative lack of data because chemical and/or enzymatic methods were used to assess and deduce the content and distribution of fructan, and the techniques used for these analyses did not allow the separation or identification of fructan. Recently, new techniques for separating fructan determining their structural composition in onions have been developed.

However, apart from the identification of the saccharide using only HPAEC, the structure of fructan in onion bulbs has been paid little attention. Therefore, we attempted to clarify the structure of fructan obtained from onion bulbs (Allium cepa L.) by high resolution 13C-NMR spectroscopy and MALDI-TOF MS of the native saccharide as well as gas-liquid chromatography of the methyl derivatives of the methanolyzed monosaccharides.

MATERIALS AND METHODS

Materials. Onion (Allium cepa L.) bulbs were harvested in October at the experimental farm of Rakuno Gakuen University and stored at −30°C. Jerusalem artichoke tubers (Helianthus tuberosus L.) and timothy (Phleum pratense L.) roots were obtained from the Hokkaido National Agricultural Experiment Station in November. Standard sugars were prepared as follows: crystalline 1-kestose (1F-β-D-fructofuranosyl)sucrose and nystose [1F(1-β-D-fructofuranosyl)sucrose] were prepared from sucrose using the Scopulariopsis brevicaulis enzyme.3) Neokestose and 1F(1-β-D-fructofuranosyl)m-6(1-β-D-fructofuranosyl)sucrose (4b: m = 0, n = 2; 4c: m = 1, n = 1; 5a: m = 3, n = 0; 5b: m = 0, n = 3; 5c: m = 2, n = 1; 5d: m = 1, n = 2; 6a: m = 4, n = 0; 6b: m = 0, n = 4; 6c: m = 3, n = 1; 6d: m = 1, n = 3; 6d: m = 2, n = 2) were isolated from asparagus roots.8,11) 1F(1-β-D-fructofuranosyl)sucrose (7a) and 1F(1-β-D-fructofuranosyl)sucrose (n ≥ 5) were prepared from Jerusalem artichoke tubers. Timothy levan was obtained from the roots of Phleum pratense L..11) Inulin was purchased from Nakalai Tesque inc. (Kyoto, Japan).

Quantitative determination of sugar. Total sugars were determined by the anthrone method.12) Reducing sugars were...
quantified using the methods described by Somogyi and Nelson.\textsuperscript{15,16}

**High performance anion exchange chromatography (HPAEC).** The saccharides, from monomer to polymer, were analyzed using a Dionex Bio LC Series (Thermo Fisher Scientific Inc., Waltham, USA) apparatus containing an HPLC carbohydrate column (CarboPac PA1, inert styrene-divinylbenzene polymer) by pulsed amperometric detection (PAD).\textsuperscript{16,17} The elution gradient was established by mixing eluent A (150 mM NaOH) with eluent B (500 mM sodium hydroxide, and concentrated in vacuo). After the precipitate was washed with boiling 70% ethanol (1.5 L) containing a small amount of calcium carbonate. This treatment was repeated three times. The resulting extract was concentrated to 200 mL in vacuo at 30–35 °C, precipitated with basic lead acetate and allowed to stand overnight. After the precipitate was removed by filtration, the filtrate was bubbled with hydrogen sulfide gas and the resulting precipitate was filtered off. The solution was degassed, neutralized with 0.5 M sodium hydroxide, and concentrated in vacuo to give a white powder corresponding to neutral soluble carbohydrates. Five grams of the sugar powder was solubilized in water and subjected to preparative paper chromatography. The paper chromatography was done using a solvent system consisting of n-propanol, ethyl acetate, and water in a 7:1:2 ratio and filter papers of Toyo No. 50 and 514A (Toyo Roshi Kaisha, Ltd., Tokyo, Japan). After triple to quintuple developments, the chromatograms were sprayed with an anisidine phosphate reagent, a mixture of 500 mg of sodium hydride and 5 mL of DMSO was stirred in a flask under a nitrogen atmosphere. To prepare the carbanion solution, a mixture of 500 mg of sodium hydride and 5 mL of DMSO was stirred in a flask under a nitrogen atmosphere at 80°C. A 0.75 mL aliquot of the latter solution was added to 1 mL of the former and stirred for 3.5–5.0 h at 20°C. Subsequently, 0.5 mL of methyl iodide was added and the solution stirred for an additional 15 h. The reaction mixture was diluted with water and extracted with chloroform. The chloroform extract was washed with water and concentrated in vacuo to give the methylated products in a syrupy residue. The permethylated saccharides were methanolysed by heating with 1.5% methanolic hydrochloric acid at 96°C for 5–20 min. The reaction mixture was treated with Amberlite IRA-410 (OH−) to remove hydrochloric acid, and evaporated in vacuo to dryness. The resulting methanolysate was dissolved in a small quantity of methanol and subjected to gas chromatography.

**Gas liquid chromatography (GLC).** For analyzing the methanolysate, GLC was carried out on a Shimadzu GC 8A gas chromatograph (Shimadzu Corporation, Kyoto, Japan) using a glass column (2.6 mm × 2 m) packed with 15% butane-1,4-diol succinate polyester on acid-washed Celite at 175°C. Flow rate of the nitrogen gas carrier was 40 mL/min.

**RESULTS AND DISCUSSION**

The fructan was obtained from 70% ethanol extract of onion bulbs using preparative paper chromatography and gel permeation chromatography with Toyopearl HW40S column. The yield of the fructan was 31 mg. The chromatograms of the neutral soluble saccharides and the fructan from onion bulbs are shown in Fig. 1. The neutral soluble saccharides gave peaks corresponding to fructooligosaccharides (1-kestose, neokestose, 4a, 4b, 4c, 5a, 5b, 5c, 5d, 6a, 6b, 6c, 6d, and 6d)) by HPAEC (elution system II) as shown in Fig. 1 (a). The fructan gave peaks corresponding to saccharides of DP (degree of polymerization) 7, 8, 9, 10, 11, 12, and 13 by HPAEC as shown in Fig. 1 (b). The retention times of the peaks were similar to those of saccharides of DP 7–13 prepared from artichoke tubers (data not shown). Saccharide signals corresponding to [M + Na]+ at m/z = 1,175, 1,337, 1,499, 1,661, 1,823, 1,985, and 2,147 were observed by MALDI-TOF MS, as shown in Fig. 2: however, the signals of the last two peaks were faint. This fructan contained no reducing sugars and released a large amount of D-fructose and a small amount of D-glucose upon hydrolysis with...
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0.5 M hydrochloric acid or yeast β-fructofuranosidase. The molar ratio of D-fructose to D-glucose in the enzyme hydrolysate was 8.7:1, as measured by HPAEC (elution system I).

Thus, the onion fructan was a mixture of polysaccharides with a DP and MW range of 7–13 and 1,152–2,124, respectively, and consisted of fructose and glucose.

The fructan was studied by 13C-NMR spectroscopy. General assignment of resonances in the fructan spectrum, measured in D2O, was tentatively carried out by comparing the observed chemical shifts with those of the reference saccharides, viz. 1-kestose, neokestose, nystose, inulin, and grass levan.

Numerous signals corresponding to carbon-1 (C-1), C-2, C-3, C-4, C-5, and C-6 of the D-fructose residues in fructan were observed at δ 61.04–61.81, 103.70–104.41, 77.46–78.10, 74.50–75.15, 81.78, and 62.85–62.96, respectively. The resonances of the fructan at δ 92.90, 71.75, 73.10, 69.76, and 72.22 resembled those of C-1, C-2, C-3, C-4, and C-5, respectively. The signal corresponding to the C-6 of the fructan residue of neokestose, as shown in Table 1: the signal corresponding to the C-6 of the

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**Fig. 1.** High performance anion-exchange chromatography of fructan prepared from onion bulbs.

(a) Neutral soluble saccharides from onion bulbs, (b) fructan from onion bulbs. Glc, glucose; Fru, fructose; Suc, sucrose; 1-K, 1-kestose; NK, neokestose; 4a, nystose; 1′(1-β-D-fructofuranosyl)n-sucrose (4b: m = 0, n = 2; 4c: m = 1, n = 1; 5a: m = 3, n = 0; 5b: m = 0, n = 3; 5c: m = 2, n = 1; 5d: m = 1, n = 2; 6a: m = 4, n = 0; 6b: m = 0, n = 4; 6c: m = 3, n = 1; 6d: m = 1, n = 3; 6d: m = 2, n = 2; 7a: m = 5, n = 0). HPAEC was carried out using elution system II.

**Fig. 2.** MALDI-TOF MS analysis of fructan prepared from onion bulbs.
D-glucose residue of fructan has not been assigned yet. On the other hand, the resonance at 6 72.22, corresponding to the C-5 of the D-glucose residue of fructan, was more sensitive to the shielding effect than those of the terminal D-glucose residue of 1-kestose, nystose, and inulin, as shown in Table 1. This indirectly suggests that the C-6 of D-glucose residue was glycosidically linked with the C-2 of the D-fructose or D-fructose oligomer of fructan. Thus, the fructan molecule contained a non-terminal D-glucose moiety as shown in Fig. 3. Furthermore, polyfructans of the levan and other saccharides are expected to help in the elucidation of their physiological and biochemical roles in onion bulbs. The structural studies of the levan molecule contained a non-terminal D-glucose moiety as shown in Fig. 3. Furthermore, polyfructans of the levan type i.e., timothy levan and bacterial levan, were also analyzed by 13C-NMR. Six strong signals (δ 60.80, 105.04, 77.20, 76.07, 81.13, and 64.20) corresponding to C-1, C-2, C-3, C-4, C-5, and C-6, respectively of the D-fructose residues of bacterial levan were observed: their chemical shifts were the same as those for timothy levan. The chemical shifts of the fructosyl residues of onion fructan differed from those of levan obtained from bacterial and plant sources.

To confirm the bond structures of onion fructan, methyl derivatives from permethylated fructan were studied using GLC. The fructan and reference saccharides were permethylated, methanolyzed, and subjected to GLC. The relative retention times (tR) of the methanolysate of the permethylated fructan and reference saccharides were shown in Table 2. The methanolysis of the onion permethylated fructan gave peaks corresponding to methyl 1,3,4,6-tetra-O-methyl-D-fructoside (tR, 1.03 and 1.25), methyl 3,4,6-tri-O-methyl-D-fructoside (tR, 2.64 and 3.94), and methyl 2,3,4-tri-O-methyl-D-glucoside (tR, 2.48: shoulder and 3.51); it gave no peaks corresponding to methyl 1,3,5-tri-O-methyl-D-fructoside. However, a very small peak corresponding to methyl 2,3,4,6-tetra-O-methyl-α-D-glucoside was faintly observed. These findings supported the structure of the fructan that was proposed on 13C-NMR data.

From these results, fructan prepared from onion bulbs was shown to consist of polymers possessing approximately 6 to 12 D-fructose residues linked by β-2,1 bonds and a non-terminal D-glucose residue bound with D-fructose residues at the C-1 and C-6 positions, i.e., 1{(1-β-D-fructofuranosyl)6-6{(1-β-D-fructofuranosyl)1-sucrose, as shown in Fig. 3. Few studies on the structure of onion bulb fructan have appeared in the literature. In the present study, onion bulb fructan was found to be a mixture of saccharides (DP 7–13), with the predominant size being DP 8–10. Recently, fructan has been the focus of intense research, because of interest in the regulation of their catabolism during growth, dormancy, and sprouting in onion bulbs. The structural studies of the saccharide are expected to help in the elucidation of their physiological and biochemical roles in onion bulbs.

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