Note

Identification of Fructo-oligosaccharides from the Bulbs of *Lycoris radiata* Herbert

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The isolation and characterization of (2→1)- β -fructosyl-sucrose series oligosaccharides in plant tissues have been reported.¹⁻³⁾ Lycorisin, fructosyl [(2→1)- β -fructosyl]₇ α -glucoside was isolated from the bulbs of *Lycoris radiata* by Mizuno *et al.*⁴⁾ In addition of the fructan they found two unknown oligosaccharides⁵⁾ containing fructose residues in the carbohydrate component. In later studies, Uchiyama⁶⁾ showed that inulin fructo-transferase (EC 2.4.1.93) activity was detected in the root of *L. radiata*, and the inulin was depolymerized very slowly by the enzymic reaction. It seems likely that potential substrates of the enzyme are present in the bulbs of *L. radiata*. During the course of investigation of fructo-oligosaccharides in the bulbs, we have isolated the 1-kestose, nystose, pentaose, and hexaose of the (2→1)- β -fructosyl sucrose series.

Bulbs of *L. radiata* were harvested at the Takawashi Experimental-Farm of Osaka Kyoiku University, in April,

1983. Standard sugars used were described previously.⁷⁾ Sliced bulbs of *L. radiata* (55 g) were extracted with 80% ethanol (500 ml) at 90°C for 2 hr. The extract was cooled and filtered to remove tissue fragments and a precipitate that formed. The filtrate was evaporated under reduced pressure at 40°C to yield a syrup (5 g), then dissolved in 70 ml of water. The method used for the separation of this extract on a charcoal-Celite column (4.7×40 cm) was described in our previous paper.⁷⁾ After the column had been washed with water (2 liters) and 5% ethanol (4 liters), sugars were eluted with 7.5% ethanol (3.2 liters) and 10% ethanol (3.2 liters) successively (Fig. 1). Each fraction indicated by a bar was combined and concentrated. Thin-layer chromatographic analysis showed that fraction A (198 mg) corresponded to 1-kestose, and fractions B (315 mg), C (225 mg), and D (251 mg) to nystose, pentaose, and hexaose respectively, though each sugar fraction was contaminated with some other sugars. A part (100 mg as syrup) of fraction A was dissolved in 4 ml of water and then put on a cellulose-powder column (2.8×30 cm)⁹⁾ with elution with 300 ml of a solvent system of 1-butanol-ethanol-water (1.5:1:1, v/v/v). Fractions B, C, and D were also purified by fractionation on a cellulose column in the same way, with the exception that the solvent system was changed to 1-butanol-ethanol-water (2:1:1, v/v/v). The results are shown in Fig. 2. After each fraction was analyzed by thin-layer chromatography, fractions containing a single component were combined as indicated, and concentrated. These sugars were pure by thin-layer chromatography. Each sample was dissolved in 2.5 ml of methanol, and sugars were precipitated by adding 9 ml of diethylether. After centrifugation (3000 rpm, 15 min), the precipitates were washed with 4 ml of diethylether and dried. Their yields were: fraction A, 31.8 mg; B, 19.1 mg;

FIG. 1. Carbon-Celite Column Chromatography of 80% Ethanol-extract of *L. radiata* Bulb.

Sample, 5 g of ethanol-extract; column, (4.7×40 cm) carbon-Celite (1:2); flow rate, 260 ml/hr. The ketohexose in each fraction was measured by the method of Roe.⁸⁾

FIG. 2. Fractionation of Fractions A, B, C, and D on a Cellulose Powder Column by Development with 1-butanol-ethanol-water.

Sample, 100 mg of each fraction; column, (2.8 × 30 cm) cellulose (Whatman CF 11 cellulose powder); flow rate, 15 ml/hr; 10 ml/fraction. The ketohexose in each 0.2 ml of fraction was measured by the method of Roe.

TABLE I. PROPERTIES OF ISOLATED FRUCTO-OLIGOSACCHARIDES

Fraction	Fructose ^a / Glucose ^b	$[\alpha]_D^{20}$ in H ₂ O (C)	Lit. $[\alpha]_D^{20}$	
A	1.93	+28.9° (0.61)	+25.4°, +29.2°	1-Kestose ^{11,12)}
B	3.19	+10.3° (0.61)	+10.6°, +10.0° + 8.9°	Nystose ^{13,2,14)}
C	4.23	- 3.6° (0.54)	- 2.3°	Pentaose ¹⁵⁾
D	5.00	-10.6° (0.25)	-10.1°	Hexaose ¹⁵⁾

^a Fructose, measured by Roe's method.

^b Glucose, measured by glucose oxidase method.¹⁶⁾

^c $[\alpha]_D^{20}$, measured with a Jasco DIP 180 type polarimeter having a 1.0 dm light path.

C, 18.3 mg; and D, 8.0 mg. None of the sugars showed reducing power (Nelson-Somogyi's reagent),¹⁰⁾ and products resulting from the hydrolysis with 0.1 N H₂SO₄ at 60°C for 100 min were confirmed by thin-layer chromatog-

raphy to be fructose and glucose. The molar ratios of ketohexose to glucose in each of the acid hydrolyzates and specific rotations of the isolated sugars are summarized in Table I. To examine the fructosidic linkages in these

FIG. 3. Thin-layer Chromatogram Showing Formation of Reaction Products from the Isolated Fructo-oligosaccharides by *A. ureafaciens* Inulin Fructotransferase.

A~D, 0.1 ml of 0.245% each sugar was incubated with 0.1 ml of *A. ureafaciens* enzyme (0.12 U) and 0.05 ml of 0.1 M acetate buffer (pH 6.0) under toluene at 30°C for 50 hr. A'~D', control, the enzyme solution was replaced by water. St, (standard sugars) D, difructose anhydride III; Su, sucrose; 1-K, 1-kestose; N, nystose; P, pentaose; H, hexaose. Chromatoplate, Kieselgel 60 F₂₅₄ (Merck); solvent system, 1-butanol-2-propanol-water (3:12:4, v/v/v);¹⁹⁾ development, ascending 10 cm, doubled; detection, naphthoresorcinol-H₂SO₄ reagent.²⁰⁾

oligosaccharides, each sample was incubated with inulin fructotransferase (formerly, inulinase II) of *Arthrobacter ureafaciens* the activity of which is exclusively on (2→1)-β-linked fructan and fructo-oligosaccharides of above DP 4 (degree of polymerization).^{7, 17)} The resulted chromatogram showed that fractions B, C, and D, but not fraction A, were susceptible to the action of the enzyme and gave difructose anhydride III (α-D-fructofuranose β-D-fructofuranose 2',1:2,3'-dianhydride)¹⁸⁾ together with sucrose, 1-kestose and nystose (followed by sucrose), respectively (Fig. 3). The results described above strongly suggested that the isolated fructo-oligosaccharides were fraction A, 1-kestose *O*-β-D-fructofuranosyl-(2→1)-*O*-β-D-fructofuranosyl α-D-glucopyranoside; B, nystose *O*-β-D-fructofuranosyl-[(2→1)-*O*-β-D-fructofuranosyl]₂ α-D-glucopyranoside; C, pentaose *O*-β-D-fructofuranosyl-

[(2→1)-*O*-β-D-fructofuranosyl]₃ α-D-glucopyranoside; and D, hexaose *O*-β-D-fructofuranosyl-[(2→1)-*O*-β-D-fructofuranosyl]₄ α-D-glucopyranoside.

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