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Fructo-oligosaccharides from *Urginea maritima* †

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ABSTRACT

Fructo-oligosaccharides from red squill (*Urginea maritima*) were isolated by precipitation with methanol, GPC on Biogel P2/P4, and reversed-phase HPLC. Structures of the tri- and tetra-saccharides were verified by the reductive cleavage method. A tetrasaccharide that contained both (2 → 1)- and (2 → 6)-linked β-D-Fru*f* residues was isolated. The higher fractions from GPC were analysed by the reductive cleavage method without prior purification by reversed-phase HPLC. The mode of biosynthesis of sinistrin is discussed.

INTRODUCTION

The fructan sinistrin, isolated from red squill (*Urginea maritima*), is utilised for renal clearance tests and, therefore, is of technical and commercial interest[1]. Sinistrin was thought originally to consist of (2 → 1)-linked β-D-Fru*f* residues with branches at positions 6 and with an α-D-Glc*p* residue attached to the reducing end of the fructose chain[2–4]. However, recent investigations revealed sinistrin to be a mixed-type β-D-fructan which comprises (2 → 1)-linked, and a relatively high proportion of (2 → 6)-linked, unbranched β-D-Fru*f* residues and an α-D-Glc*p* residue linked as in neokestose[5]. It was not clear if the differently linked D-Fru*f* residues occurred in the same or different molecules.

The aim of the present work was to isolate and characterise the low molecular weight fructo-oligosaccharides from dried slices of red squill and to ascertain whether (2 → 1)- and (2 → 6)-linked β-D-Fru*f* residues are present in the same molecule.

The low molecular weight fructans of wheat[6–8], barely[7–9], onion[7], and Jerusalem artichoke[7] have been investigated by gel-permeation chromatography (GPC) fol-

† Studies of Fructans, Part III. For Part II, see W. Praznik, T. Spies, and A. Hofinger, *Carbohydr. Res.*, 235 (1992) 231–238.

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lowed by reversed-phase HPLC. In contrast to Bancal[7], we have used the reductive cleavage method for analysis of the structure of β-D-fructans[5,10,11] because it enables a better discrimination of (2 → 1) and (2 → 6) linkages.

EXPERIMENTAL

Materials.—Dried slices from red squill were obtained from the Laevosan Co. Biogel P2 (−400 mesh) and P4 (−400 mesh) were purchased from Bio-Rad Laboratories and Nucleosil 120 7 C-18 from Macherey & Nagel.

Isolation of the fructans.—The above dried slices were homogenised and an aqueous solution was freeze-dried. A solution of the residue (300 g) in aq 80% MeOH (1 L) was left overnight at 4°C, then centrifuged at 5000 rpm, and the supernatant was concentrated in vacuo to 40 mL.

The carbohydrates were precipitated by the addition of acetone (160 mL), collected by centrifugation at 5000 rpm, dissolved in water (40 mL), precipitated with acetone (160 mL), and isolated by centrifugation at 5000 rpm, and a solution in water (100 mL) was freeze-dried to yield a pale-red powder (30 g).

A solution of 500 mg of the crude extract in distilled water (3 mL) was centrifuged at 5000 rpm and then eluted from columns of Biogel P2 (−400 mesh; 90 × 2.5 cm) and Biogel P4 (−400 mesh; 87 × 2.5 cm) in sequence with distilled water and degassed water at 0.4 mL/min. A Waters RI-detector R-403 was used and fractions (5 mL) were collected with an LKB fraction collector. A Hitachi 561 Recorder was used. The fractions 105–108 (106), 99–103 (101), 95–98 (96), 90–93 (92), 83–89 (85), 60–70 (65), and 45–50 (47) were combined, freeze-dried, and designated as in parentheses.

Each fraction was analysed by TLC (1-butanol–1-propanol–EtOH–water, 2:3:3:2) on silica gel (Whatman; detection with thymol) with inulo-oligosaccharides from Jerusalem artichoke as standards.

A solution of each fraction in water (500 μL) was subjected to reversed-phase HPLC on a column (300 × 10 mm i.d.) of Nucleosil 120-7 C18, using filtered and degassed water at 2.5 mL/min in a system that consisted of Rheodyne Model 7125 sample injector, an LKB-2150 pump, an ALTEX 156 differential refractometer, and a Hewlett–Packard 3396 II integrator. The fractions (1 mL) were collected by hand.

Determination of structure.—Methylation[12] and reductive cleavage[13] were carried out as described. GLC–MS was performed on a Carlo Erba Mega series HRGC 5300 gas chromatograph and a Finnigan Mate ion-trap mass spectrometer. A DB 1701 capillary column (0.25-μm film thickness, 25 m × 0.25 mm i.d.) was used with He as the carrier gas. The temperature program was 100 → 250°C at 4°C/min. For quantitative analysis, an HP 5880 gas chromatograph and a Permabond OV-1701 capillary column (0.25-μm film thickness, 30 m × 0.25 mm i.d.) (Macherey & Nagel) were used with the above temperature program and N_2 as the carrier gas.

RESULTS AND DISCUSSION

Fig. 1 shows the results of the GPC on the Biogel P2/P4 system of the extract from red squill. There is only a relatively small proportion of low molecular weight fractions, which is in accord with the results of Nitsch et al.[4].

TLC revealed fraction 120 to be glucose and fructose, and fraction 111 to be sucrose. Semi-preparative reversed-phase HPLC of fraction 106 gave (Fig. 2) two major peaks, with retention times 14.1 (106/1) and 18.8 min (106/2), that were identified as 1-kestose [α-D-Glc*p*-(1 ↔ 2)-β-D-Fru*f*-(1 ← 2)-β-D-Fru*f*] and neokestose [β-D-Fru*f*-(2 → 6)-α-D-Glc*p*-(1 ↔ 2)-β-D-Fru*f*] by the reductive cleavage method. 6-Kestose [α-D-Glc*p*-(1 ↔ 2)-β-D-Fru*f*-(6 ← 2)-β-D-Fru*f*], which should elute prior to 1-kestose[14], was not collected.

Likewise, fraction 101 (Fig. 3) gave 6 major peaks that were designated fractions 101/1–101/6. TLC revealed that each fraction contained a single component with the following R_f values: 101/1 0.39, 101/2 0.41, 101/3 0.47, 101/4 0.49, 101/5 0.44, and 101/6 0.44.

The compounds in fractions 101/1–101/6 were analysed by the reductive cleavage method, and Table I shows the molar percentages calculated from the corrected peak areas[5], linkages of the β-D-Fru*f* and α-D-Glc*p* residues, the calculated dp, and the number of the differently linked β-D-Fru*f* and α-D-Glc*p* residues per molecule. For the calculation of the dp, it was assumed that only one α-D-Glc*p* per molecule was present. The HPLC did not separate the components completely. Fig. 3 shows that the sample was heterogeneous, and only the compounds in the major peaks were isolated. Fraction 101/1 was identified as the tetrasaccaride bifurcose (β-D-Fru*f*-(2 → 6)[α-D-Glc*p*-(1 ↔ 2)]-β-D-Fru*f*-(1 ← 2)-β-D-Fru*f*)[7,15]. Fraction 101/2 was a tetrasaccharide that contained α-D-Glc*p* residues and β-D-Fru*f* residues together with a 1- and a 6-substituted β-D-Fru*f* residue, thus confirming the presence of two types of linked β-D-Fru*f* residues in

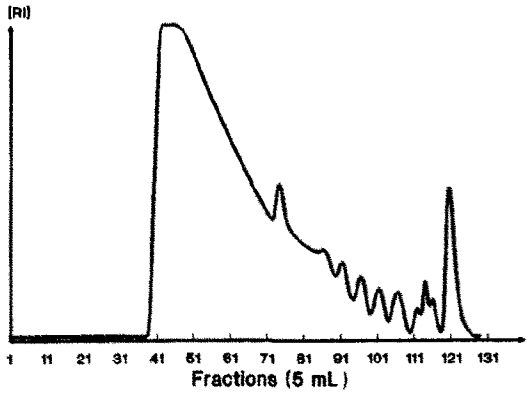


Fig. 1. GPC on Biogel P2 and P4 of the extract from red squill (see Experimental).

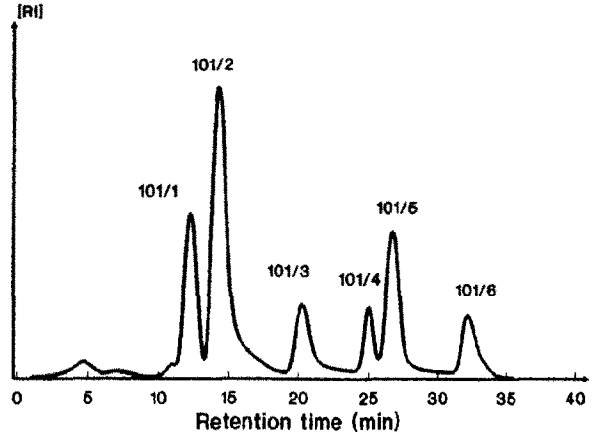


Fig. 2. HPLC of fraction 106 from Fig. 1 on Nucleosil 120-7 C-18 (see Experimental).

the same molecule. There are two possible structures for fraction 101/2 with the 1- or the 6-substituted β-D-Fru*f* residue linked to the α-D-Glc*p* residue. Since no 6-kestose was detected, structure **1** is the most probable because it can be derived from 1-kestose by transfructosylation.

Two stuctures are possible for each of the tetrasaccharides in fractions 101/3, 101/4, and 101/6. These alternatives always occur if a 6-linked α-D-Glc*p* residue is part of the molecule because the β-D-Fru*f* chain can be elongated at either end. The structures **2**–**4** for fractions 101/3, 101/4, and 101/6, respectively, are

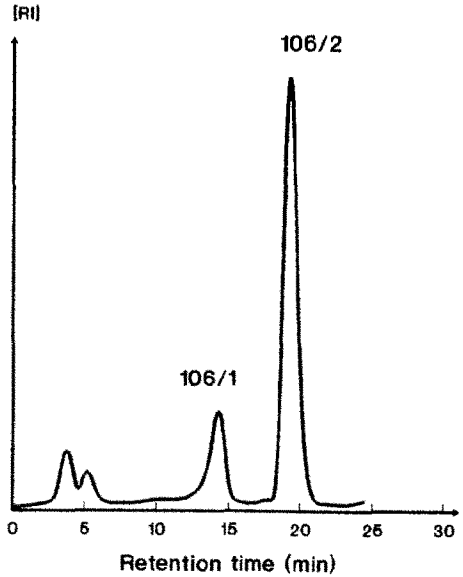
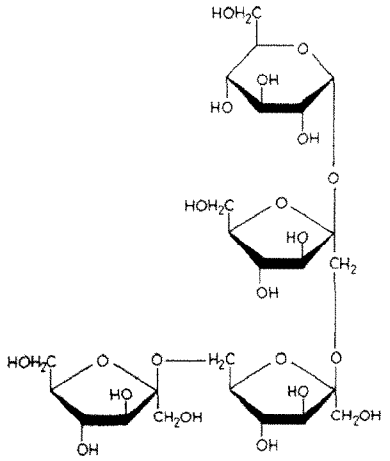


Fig. 3. HPLC of fraction 101 from Fig. 1 on Nucleosil 120-7 C-18 (see Experimental).

TABLE I
Percentage (%) and number (n) per molecule of β-D-Fru$f$ and α-D-Glc$p$ residues in the fractions obtained by the reductive cleavage [a] method

Fraction		106/1		106/2		101/1		101/2		101/3	
		%	n	%	n	%	n	%	n	%	n
β-D-Fru$f$	terminal	30 ±0.09	1.0±0.03	70 ±2.1	2.1±0.06	44 ±1.3	2.0±0.06	29 ±0.8	1.2±0.03	46 ±1.2	1.9±0.05
	1-linked	33 ±3.2	1.1±0.11			3 ±0.3	0.2±0.01	21 ±2.1	1.0±0.03		
	6-linked	6 ±0.6	0.2±0.02	9 ±0.3	0.3±0.01	7 ±0.5	0.2±0.02	21 ±2.1	1.0±0.03	30 ±0.3	1.1±0.11
	1,6-linked					23 ±0.7	1.1±0.03	5 ±0.4	0.3±0.01		
α-D-Glc$p$	terminal	28 ±0.8	0.9±0.03			23 ±0.6	1.0±0.03	22 ±0.6	0.9±0.03		
	6-linked	3 ±0.1	0.1±0.01	29 ±0.8	1.0±0.03			2 ±0.1	0.1±0.01	24 ±0.7	1.0±0.03
dp		3.3		3.4		4.3		4.2		4.2	
Fraction		101/4		101/5		101/6		96		92	
		%	n	%	n	%	n	%	n	%	n
β-D-Fru$f$	terminal	38 ±1.1	1.5±0.05	23 ±0.7	1.1±0.03	45 ±1.3	1.9±0.06	39 ±1.2	2.2±0.07	31 ±0.9	1.7±0.05
	l-linked	30 ±3.0	1.2±0.04	50 ±5.0	2.3±0.06	28 ±2.8	1.1±0.11	25 ±2.5	1.4±0.14	27 ±2.7	1.5±0.15
	6-linked	3 ±0.3	0.1±0.01	3 ±0.3	0.2±0.01	2 ±0.2	0.1±0.01	12 ±1.2	0.7±0.07	14 ±1.4	0.8±0.08
	1,6-linked	3 ±0.1	0.1±0.01	2 ±0.1	0.1±0.01			5 ±0.2	0.3±0.01	11 ±0.3	0.6±0.02
α-D-Glc$p$	terminal	3 ±0.1	0.1±0.01	22 ±0.7	1.0±0.03			9 ±0.3	0.5±0.02	8 ±0.2	0.5±0.02
	6-linked	23 ±0.7	0.9±0.03			25 ±0.7	1.0±0.03	10 ±0.3	0.5±0.02	9 ±0.7	0.5±0.02
dp		3.8		4.5		4.0		5.5		5.8	
Fraction		85		65		47					
		%	n	%	n	%	n				
β-D-Fru$f$	terminal	31 ±0.9	1.9±0.06	25 ±0.8	3.3±0.10	22 ±0.7	6.7±0.20				
	1-linked	28 ±2.8	1.8±0.18	29 ±2.9	3.8±0.38	30 ±3.0	9.0±0.90				
	6-linked	14 ±1.4	0.9±0.09	19 ±1.9	2.5±0.25	21 ±2.1	6.4±0.64				
	1,6-linked	12 ±0.8	0.7±0.02	19 ±0.6	2.5±0.07	23 ±0.7	7.0±0.21				
α-D-Glc$p$	terminal	6 ±0.2	0.4±0.13	2 ±0.1	0.3±0.01						
	6-linked	9 ±0.3	0.6±0.02	6 ±0.2	0.7±0.02	3 ±0.1	0.9±0.03				
dp		6.8		13		32					

[a] See Figs. 1–3.



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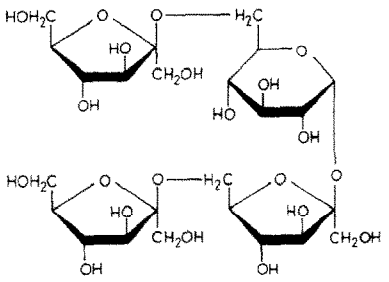

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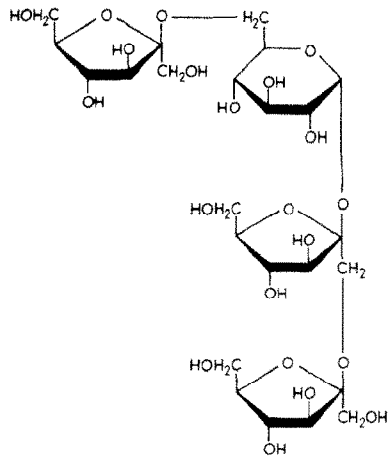

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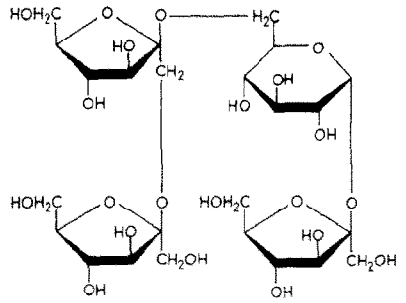

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possible but have not been proved. Fraction 101/5 was identified as nystose [α-D-Glc*p*-(1 ↔ 2)-[β-D-Fru*f*-(1 ← 2)]$_2$-β-D-Fru*f*].

The biosynthesis of sinistrin seems to start with sucrose, as in all plant fructans. The so called 6-G fructosyltransferase[16] synthesises neokestose (fraction 106/1) and the enzyme sucrose:sucrose 1^F-β-D-fructosyltransferase (SST, EC 2.4.1.99) synthesises 1-kestose (fraction 106/2). The tetrasaccharides are synthesised by the action of fructan:fructan 1^F-β-D-fructosyltransferase (FFT EC 2.4.1.100)[17] and possibly 6-FFT. At this stage, all possible saccharides are formed. However, for the higher fractions, the ratio of (2 → 1)- and (2 → 6)-linked β-D-Fru*f* residues and the degree of branching approach constant values (Fig. 4). The fraction with dp ~ 13 has a structure which is similar to that of sinistrin with dp 30 (Table 1)[5].

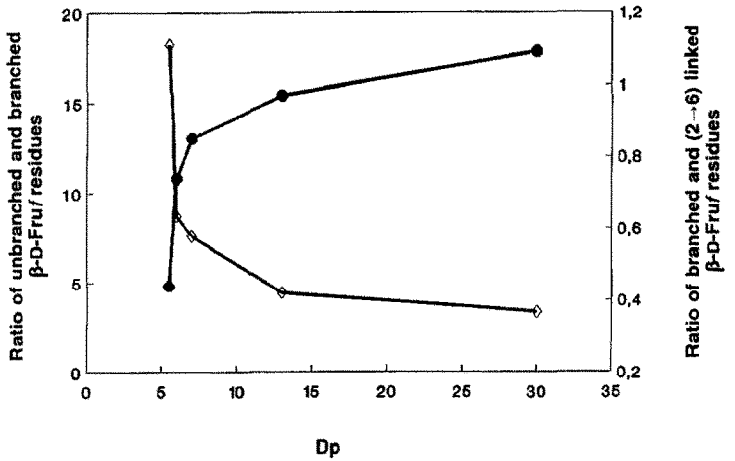

Fig. 4. Relationship of the dp with the ratio of unbranched and branched α-D-Glc*p* and β-D-Fru*f* residues (–◇–) and the ratio of branched and (2 → 6)-linked β-D-Fru*f* residues (–●–) in the fractions of sinistrin obtained by GPC.

The above results confirm the model presented in the preceding paper[5] in that (*a*) (2 → 1)- and (2 → 6)-linked β-D-Fru*f* residues occur in the same molecule and (*b*) sinistrin seems to have a regular structure.

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