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Fructo-oligosaccharides from the stems of *Triticum aestivum* *

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ABSTRACT

Fructo-oligosaccharides, extracted with hot water from wheat stems before flowering, were fractionated by gel-permeation chromatography on Biogel P2. The tri-/tetra- and penta-saccharide fractions were purified by HPLC and analysed by the reductive cleavage method. The trisaccharides 1-kestose and 6-kestose, the branched tetrasaccharide bifurcose, and the (2 → 6)-linked pentasaccharide were identified. The fractions of higher molecular weight were also investigated and confirmed the branched-levan structure of this fructan.

INTRODUCTION

The fructans of cereal grasses consist mainly of chains of (2 → 6)-linked β-D-Fru*f* residues with branches at positions 1 and a minor proportion of (2 → 1)-linked residues[1,2]. Early investigations[2–5] on wheat fructans led to the proposal of a levan [i.e., a (2 → 6)-linked] backbone with branches for the wheat-flour fructan. Levan- and inulin-type [i.e., (2 → 1)-linked] fructans[6] were found in wheat meal. Inulin also appears in the flowers, but not in the stems where only (2 → 6)-linked fructans were detected[2].

Gel-permeation chromatography of the fructans from developing wheat leaves and methylation analysis of the fractions showed[7] the trisaccharide fraction to contain a high percentage of 1-kestose [α-D-Glc*p*-(1 → 2)-β-D-Fru*f*-(1 ← 2)-β-D-Fru*f*] and little 6-kestose [α-D-Glc*p*-(1 → 2)-β-D-fru*f*-(6 ← 2)-β-D-Fru*f*]. In contrast to such grasses as tall fescue, rye grass, and timothy grass, no neokestose [β-D-Fru*f*-(2 → 6)-α-D-Glc*p*-(1 → 2)-β-D-Fru*f*] was detected[8,9]. Bancal et al.[10] also isolated and characterised fructo-oligosaccharides from wheat-leaf blades by hydrolysis with a fructan exohydrolase. Nystose, bifurcose, and some neokestose-con-

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taining tetrasaccharides were detected together with several penta- and hexa-saccharides. The (2 → 1)-linked oligosaccharides preponderated. In the fractions of higher molecular weight, branched (2 → 6)-linked fructans preponderated, although substantial proportions of (2 → 1)-linked β-D-Fru*f* residues were detected[7].

We now report on the fructans isolated from wheat stems before flowering. In this early stage of plant development, a large proportion of fructans is present in the stems.

EXPERIMENTAL

Materials.—Homogenised and freeze-dried, immature wheat stems without leaf blades were a gift from Dr. M. Krumm (University of Stuttgart). Biogel P2 (−400 mesh, superfine) was purchased from Biorad-Laboratories, and Nucleosil 120-7 C-18 from Macherey & Nagel.

Isolation of the fructans.—The above wheat stems (5 g) were extracted with boiling water (50 mL) for 1 min. The extract was centrifuged and the supernatant solution was freeze-dried. The residue (500 mg) was dissolved in water (10 mL), precipitated with acetone (40 mL), centrifuged, and dried in vacuo to yield the crude fructan (400 mg).

A solution of the crude fructan in distilled water (3 mL) was centrifuged at 3000*g*, then subjected to gel filtration (GPC) on a column (90 × 2.5 cm) of Biogel-P2 (−400 mesh, super-fine) by elution with degassed water at 0.4 mL/min. The eluate was monitored with a Waters RI-detector R-403 and the fractions (5 mL) were collected with an LKB fraction collector, using an Hitachi 561 Recorder. Fractions 61–66 (GPC 64), 55–59 (GPC 57), 51–53 (GPC 52), 47–49 (GPC 48), 44–46 (GPC 45), 41–42 (GPC 42), 39–40 (GPC 40), 37–38 (GPC 38), and 30–36 were combined and freeze-dried.

Each GPC fraction was analysed by TLC [1-butanol–1-propanol–EtOH–H_2O (2:3:3:2) and detection with thymol] on silica gel (Whatman) with inulo-oligosaccharides from Jerusalem Artichoke as standards.

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

^{13}C NMR spectroscopy.—The spectra (75.47 MHz) were obtained for solutions (10 mg/0.5 mL) in D_2O at 20° with a Bruker AC 300 F spectrometer. Chemical shifts (δ) are expressed relative to that of external 1,4-dioxane.

Determination of structure.—Methylation[11] and reductive cleavage[12] were carried out as described. GLC–MS was performed on a Carlo Erba Mega series HRGC 5300 gas chromatograph and an ion-trap mass spectrometer from Finnigan Mate. A DB 1701 capillary column (0.25-μm film thickness, 25 m × 0.25 mm i.d.)

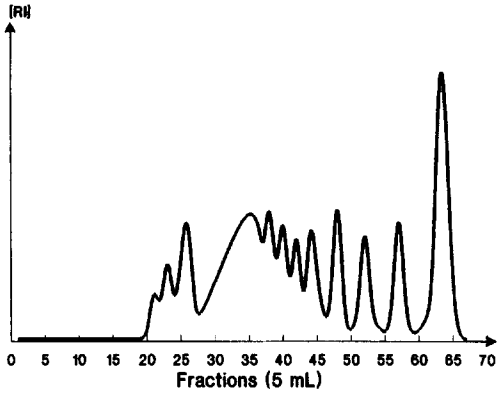


Fig. 1. Gel-permeation chromatography on Biogel P2 of the extract from wheat stems (see Experimental).

was used with He as the carrier gas. The temperature program was 100 → 250° at 4°/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

When the crushed and freeze-dried wheat-plant material was extracted with hot water and the products were fractionated on Biogel P2, a high percentage of small oligosaccharides was revealed (Fig. 1), as reported in the literature[7]. TLC of the components in each peak (Fig. 2), with inulo-oligosaccharides from Jerusalem artichoke as references, indicated fraction GPC 64 to be a monosaccharide and GPC 57 to be sucrose. The trisaccharide fraction (GPC 52) contained a component with the R_F value (0.52) of 1-kestose. The other component had R_F 0.48 and probably was 6-kestose, which has an R_F value on silica gel lower[13] than that of 1-kestose.

GPC 48 contained components with R_F values of 0.46 and 0.40 for inulopentaose. Fractions GPC 45, 40, and 38 each contained more than one component that could not be separated clearly by TLC.

Fractions GPC 52 and 48 were subjected to reversed-phase HPLC and the results for GPC 52 (Fig. 3) revealed two major peaks at 9.9 (52/1) and 14 min (52/2), and those for GPC 48 (Fig. 4) revealed 3 major peaks of which the components (48/1 and 48/2) were studied further. The purified oligosaccharides were analysed by the reductive cleavage method[14] (methylation followed by treatment with triethylsilane in the presence of trimethylsilyl trifluoromethanesulfonate). This method can discriminate between (2 → 1)- and (2 → 6)-linked β-D-Fru*f* residues unlike methylation analysis[14–16]. The 1- and 6-substituted β-D-Fru*f*

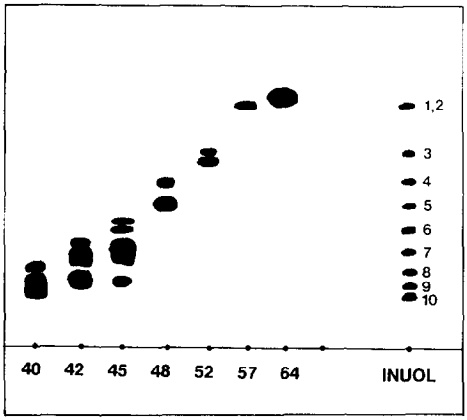


Fig. 2. TLC of the fractions from Fig. 1 and reference inulo-oligosaccharides of dp 1–10.

residues give rise to the same D-mannitol derivative but different D-glucitol derivatives (see preceding paper[16]).

Thus, fraction 52/1 was identified as 6-kestose, and fraction 52/2 as 1-kestose (Table I), in the ratio $> 4:1$.

Fraction 48/1 was identified as the unbranched pentasaccharide [β-D-Fru*f*-(2 → 6)]$_3$-β-D-Fru*f*-(2 → 1)-α-D-Glc*p* (**1**) and fraction 48/2 as the branched tetrasaccharide β-D-Fru*f*-(2 → 6)[α-D-Glc*p*-(1 → 2)]-β-D-Fru*f*-(1 ← 2)-β-D-Fru*f* (**2** bifurcose). This tetrasaccharide was described first by Schlubach and Lederer[2]. The fractions of higher molecular weight in Fig. 1 were not resolved by HPLC and were analysed only by the reductive cleavage method. Table I lists the various β-D-Fru*f* residues and their molar percentages on the assumption that only one α-D-Glc*p* residue is present per molecule. Normally, the difference between the numbers of

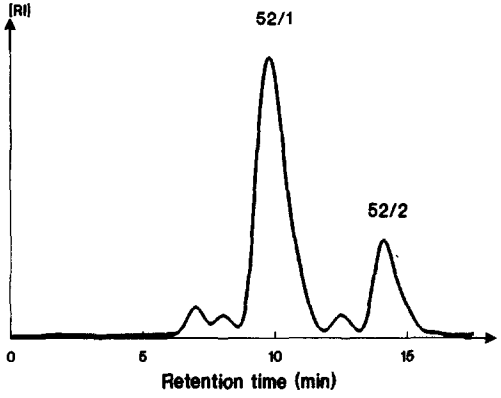


Fig. 3. HPLC of fraction 52 from Fig. 1 on Nucleosil 120-7 C-18 (see Experimental).

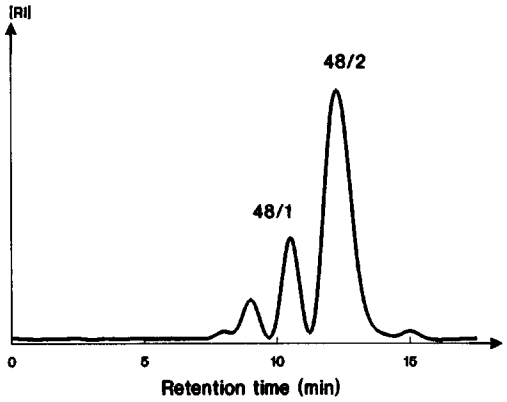


Fig. 4. HPLC of fraction 48 from Fig. 1 on Nucleosil 120-7 C-18 (see Experimental).

the terminal and the branched β-D-Fru*f* residues per molecule should be 1, and values of 0.9–1.1 indicated that there was no loss during reductive cleavage.

The NMR spectra of 6-kestose have been analysed in detail[17] and the ^{13}C data are of value for the analysis of low molecular weight levans. The resonance of C-1 is shifted upfield to δ 93.2 in comparison with that (δ 93.4) of 1-kestose[17–21]. The C-1 resonance of the terminal α-D-Glc*p* residue of the β-D-fructan of *Pucinella peisonis* was found[16] at δ 93.0. By using these data, it is possible to decide whether a terminal α-D-Glc*p* residue is linked to a β-D-Fru*f* residue that is substituted at O-1 or O-6. The other ^{13}C resonances of the α-D-Glc*p* residue are identical to these of the α-D-Glc*p* residue of 1-kestose. In the region δ 61–63, there were three signals assigned to C-6 of α-D-Glc*p* (δ 61.0), C-1 of terminal β-D-Fru*f* (δ 61.4)[20] and C-1 of 6-linked β-D-Fru*f* (δ 62.4). The linkage to α-D-Glc*p* and the 6-substitution shift the C-1 signal downfield by 1.1 ppm compared to that of C-1 in the 1-linked β-D-Fru*f* residue of 1-kestose. In longer chains of (2 → 6)-linked β-D-Fru*f* residues, the C-1 signal is shifted upfield to δ 60.8, as found in the β-D-fructans of *Agropyron repens*[16,21] and *Pucinella peisonis*[16].

Normally, the C-2 signal of (2 → 6)-linked D-Fru*f* is expected to have $\delta > 105$. However, for 6-kestose, the C-2 resonances of the two β-D-Fru*f* residues could not be separated at δ 104.9.

The signals at δ 75.9, 81.9, and 64.1 were assigned[17–19,21–23] to C-4,5,6, respectively, of the (2 → 6)-linked β-D-Fru*f*. Table II shows the ^{13}C NMR data for 6-kestose.

It is concluded that the main trisaccharide in developing wheat stems is 6-kestose, which is present in four times the concentration of 1-kestose. This conclusion contrasts with the finding of much higher proportions of 1-kestose than of 6-kestose in developing wheat leaves[7] and other grasses such as *Lolium temulentum*[8] and barley[9]. It is interesting that pentasaccharide **1** was detected in low porportion in leaf blades[10], whereas it was found here in developing stems in a

TABLE I

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

Fraction		52/1		52/2		48/1		48/2			
		%	n	%	n	%	n	%	n		
β-D-Fru$f$	terminal	32±1.0	1.1±0.03	33±1.0	1.0±0.03	22±0.7	1.1±0.03	42±1.3	2.0±0.06		
	1-linked	2±0.2	0.1±0.01	33±3.3	1.0±0.1			4±0.4	0.2±0.02		
	6-linked	33±3.3	1.0±0.10	1±0.1	0.1±0.01	57±0.6	2.9±0.29	8±0.8	0.3±0.03		
	1,6-linked							23±0.7	1.1±0.03		
α-D-Glc$p$	terminal	31±0.9	1.0±0.03	33±1.0	1.0±0.03	20±0.6	1.0±0.03	20±0.6	1.0±0.03		
Dp		3.1		3.0		5.1		4.4			
Fraction		45		42		40		38		30–36	
		%	n	%	n	%	n	%	n	%	n
β-D-Fru$f$	terminal	34±1.0	1.9±0.06	36±1.1	2.7±0.08	32±0.9	2.7±0.08	31±0.9	3.2±0.10	30±0.9	3.5±0.10
	1-linked	8±0.8	0.4±0.04	9±0.9	0.7±0.07	7±0.7	0.6±0.06	8±0.8	0.9±0.09	10±0.1	1.1±0.11
	6-linked	23±2.3	1.3±0.13	21±0.2	1.7±0.17	28±0.3	2.4±0.24	28±0.3	3.0±0.30	28±0.3	3.2±0.32
	1,6-linked	18±0.5	1.0±0.03	20±0.6	1.6±0.05	22±0.7	1.8±0.06	24±0.7	2.8±0.09	24±0.7	2.6±0.08
α-D-Glc$p$	terminal	17±0.5	1.0±0.03	14±0.4	1.0±0.03	11±0.3	1.0±0.03	9±0.3	1.0±0.03	8±0.2	1.0±0.01
Dp		5.7		7.6		8.7		10.4		11.3	

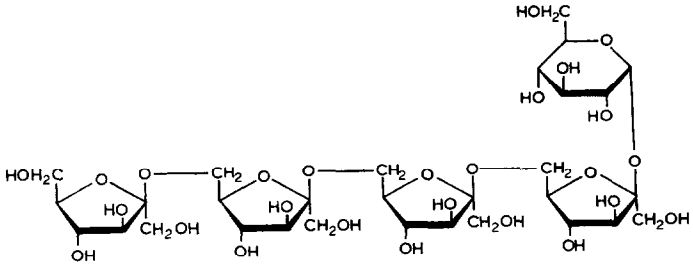
[a] See Figs. 1, 3, and 4.

TABLE II

Chemical shift assignments (δ) for the ^{13}C NMR spectrum of 6-kestose

	α-D-Glc*p*-(1 →	→ 6)-β-D-Fru*f*-(2 →	β-D-Fru*f*(2 →
C-1	93.2	62.4	61.4
C-2	72.1	104.9	104.9
C-3	73.7	77.4	77.8
C-4	70.4	75.5	75.9
C-5	73.5	81.4	82.3
C-6	61.0	64.1	63.7

relatively large proportion. The only tetrasaccharide detected was bifurcose (**2**) and no nystose was found. Schlubach and Lederer[2] proposed that every second (2 → 6)-linked β-D-Fru*f* residue in wheat fructan is branched at position 1, but the detection of the unbranched pentasaccharide **1** indicates a more-complex structure for the wheat-stem fructans[10]. These results are confirmed also by those of Carpita et al.[21], who investigated wheat stems at different stages of development by methylation analysis. (2 → 1)-Linked β-D-Fru*f* residues were also found in the fractions of higher molecular weight, and the determination of the structures of the fractions with dp 5–8 may indicate whether or not in a fructan series there are two kinds of linkages, as in sinistrin[16].



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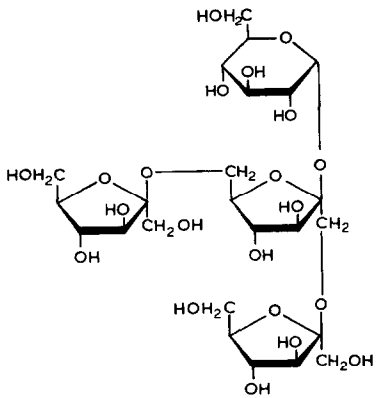

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