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The structure of the fructan sinistrin from *Urginea maritima* *

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ABSTRACT

The structure of sinistrin from red squill (*Urginea maritima*) was determined by methylation analysis and ^{13}C NMR spectroscopy, using the fructans from *Pucinella peisonis* and quack-grass (*Agropyron repens*) as reference substances. Application of the reductive cleavage method showed that, of the β-D-fructofuranosyl residues in sinistrin, 33% were 1-linked, 19% were 6-linked, 25% were terminal, and 19% were 1,6-linked. The average dp was 31 and, of the 3.24% of α-D-glucopyranosyl residues, 0.54% were terminal and 2.70% were 6-substituted. The fructan of quack grass was also highly branched with a (2 → 6)-linked backbone, terminal α-D-glucopyranosyl residues, and a dp of ~ 45. The fructan from *Pucinella peisonis* was slightly branched, with a dp of ~ 10 and a (2 → 6)-linked backbone.

INTRODUCTION

Fructans occur as a wide range of oligo- and poly-saccharides[1], as reserve plant carbohydrates in dicotyledons, as inulin[2] [(2 → 1)-β-D-fructan], and in monocotyledons mainly as (2 → 1), (2 → 6)-β-D-fructans.

Sinistrin, a fructan first isolated[3] from the bulbs of red squill (*Urginea maritima*), was thought[4,5] to consist of (2 → 1)-linked β-D-fructofuranosyl residues with branching points at C-6 and an α-D-glucopyranose residue on the reducing end of the fructan chain, and therefore related to inulin. Nitsch et al.[6] applied size-exclusion chromatography to sinistrin and obtained several fractions, for which the average molecular weight was 2500 and the highest was 16000. Small-angle X-ray scattering experiments showed sinistrin to have a more spherical structure in solution than inulin[7]. Sinistrin is used for the determination of renal clearance, since it is filtered in the kidney through the glomeruli only, and neither reabsorbed nor secreted by the cells of the tubuli[8].

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* Studies of Fructans, Part I.

In investigating the structure of sinistrin, inulin, a (2 → 1)-β-D-fructan, and a fructan from *Pucinella peisonis*, which should represent (2 → 6)-β-D-fructans[9], were chosen as standards. The structure of a fructan from quack grass[10] (*Agropyron repens*) was investigated also as a standard for the (2 → 6)-β-D-fructans.

EXPERIMENTAL

Materials.—Sinistrin, a commercial product (Inutest®), and the fructan from quack grass were kindly provided by the Laevosan Company (Linz). Inulin was prepared from Jerusalem artichoke[11].

Isolation of the fructan from Pucinella peisonis.—The thickened basal parts (240 g) of the leaf sheets of *P. peisonis*[9] were washed, then cut, and the carbohydrates were extracted with hot water. The extract was filtered through a cheese cloth and concentrated to 20 mL. Acetone (80 mL) was added, the solution was centrifuged at 5000 rpm, the precipitate was dissolved in water, again precipitated with acetone, and centrifuged, and a solution in water was freeze-dried to store the fructan (2.32 g) as a pale-brown powder. For NMR investigations, a solution of the fructan (200 mg) in water (20 mL) was decolorised with charcoal, filtered, and freeze-dried (yield 40 mg).

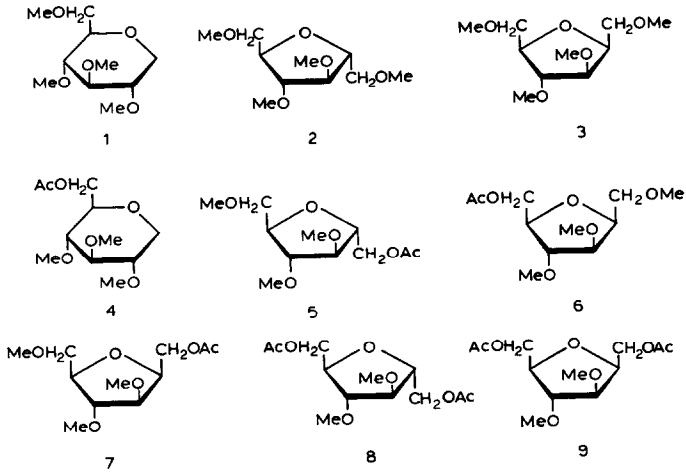
^{13}C NMR spectroscopy.—The spectra (75.47 MHz) were obtained for a solution of each fructan (10 mg) in D_2O (0.5 mL) at 20° with a Bruker AC 300 F spectrometer. Chemical shifts are expressed relative to that of external 1,4-dioxane.

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

RESULTS

According to Rolf and Gray[14], application of the reductive cleavage method (methylation followed by treatment with triethylsilane in the presence of trimethylsilyl trifluoromethanesulfonate) should convert the terminal α-D-Glc*p* residues into 1,5-anhydro-2,3,4,6-tetra-*O*-methyl-D-glucitol (**1**), terminal β-D-Fru*f* residues into 2,5-anhydro-1,3,4,6-tetra-*O*-methyl-D-mannitol (**2**) and 2,5-anhydro-1,3,4,6-tetra-*O*-methyl-D-glucitol (**3**), (2 → 1)-linked β-D-Fru*f* residues into 1-*O*-acetyl-2,5-anhydro-3,4,6-tri-*O*-methyl-D-mannitol (**5**) and 1-*O*-acetyl-2,5-anhydro-3,4,6-tri-*O*-methyl-D-glucitol (**7**), (2 → 6)-linked β-D-Fru*f* residues into **5** and 6-*O*-acetyl-2,5-anhydro-1,3,4-tri-*O*-methyl-D-glucitol (**6**), and 1,6-disubstituted β-D-Fru*f* residues

into 1,6-di-*O*-acetyl-2,5-anhydro-3,4-di-*O*-methyl-D-mannitol (**8**) and 1,6-di-*O*-acetyl-2,5-anhydro-3,4-di-*O*-methyl-D-glucitol (**9**).



The methylated, reduced, and acetylated samples were analysed quantitatively by GLC and the integrated peak areas were corrected using the effective-carbon-response method[15]. Since there should be only one α-D-Glc*p* residue per inulin molecule, its proportion should reflect the average chain length.

As expected, inulin gave **1–3**, **5**, and **7**, the mass spectra of which were identical to those in the literature[14,16]. Only small proportions of **8** and **9** were detected, so that only every second molecule should have one branched β-D-Fru*f* residue.

The fructan from quack grass gave **1–3**, **5**, **6**, a little **7**, **8**, and **9** (Fig. 1). This β-D-fructan appears to be mainly (2 → 6)-linked with a high proportion of branched residues. The formation of **7** indicates that (2 → 1)-linked β-D-Fru*f* residues were present. The peak 590 in Fig. 1 was an artefact, probably the 2,6-anhydroglucitol derivative from **8** and **9**, and was added to the areas of **8** and **9** since it had the same molecular weight. The proportion of this artefact probably depends on the amount of water in the reaction mixture. When water was excluded during the derivatisation, the proportion of the artefact decreased to ~ 5% of **8** plus **9**. The fructan from *P. peisonis* gave **1–3**, **5**, **6**, and minor proportions of **8** and **9** (Fig. 2), thus confirming the presence of mainly (2 → 6)-linked β-D-Fru*f* residues with no (2 → 1) linkages.

The sinistrin from red squill gave **1–3** and **5–9** (Fig. 3). In contrast to the fructan from quack grass, the proportion of (2 → 1)-linked β-D-Fru*f* residues was higher than that of the (2 → 6)-linked residues. The (2 → 1)- and (2 → 6)-linked β-D-Fru*f* residues give the same mannitol derivative (**5**) but different glucitol derivatives (**7** and **6**). The mannitol/glucitol ratio (4.1 ± 0.2) remains constant for inulin, where only (2 → 1)-linked β-D-Fru*f* residues were detected. Thus, (7 + 4.1

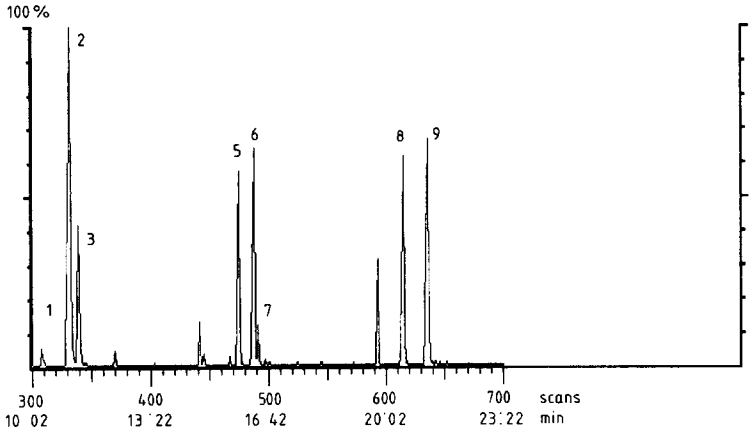


Fig. 1. GLC (see Experimental) of the products of the reductive cleavage of the fructan from quack grass: **1**, 1,5-anhydro-2,3,4,6-tetra-*O*-methyl-D-glucitol; **2**, 2,5-anhydro-1,3,4,6-tretra-*O*-methyl-D-mannitol; **3**, 2,5-anhydro-1,3,4,6-tetra-*O*-methyl-D-glucitol; **5**, 1-*O*-acetyl-2,5-anhydro-3,4,6-tri-*O*-methyl-D-mannitol; **6**, 6-*O*-acetyl-2,5-anhydro-1,3,4-tri-*O*-methyl-D-glucitol; **7**, 1-*O*-acetyl-2,5-anhydro-3,4,6-tri-*O*-methyl-D-glucitol; **8**, 1,6-di-*O*-acetyl-2,5-anhydro-3,4-di-*O*-methyl-D-mannitol; **9**, 1,6-di-*O*-acetyl-2,5-anhydro-3,4-di-*O*-methyl-D-glucitol.

$\times$ **7**) gives the proportion of (2 → 1)-linked residues and (**5** − 4.1 $\times$ **7**) is added to **6** to give the proportion of (2 → 6)-linked residues. Therefore, the ratio of (2 → 1) and (2 → 6) linkages can be estimated approximately, using these proportions with an average error of ~ 10%. In sinistrin, the α-D-Glc$p$ residue is linked not only as in sucrose, but with a β-D-Fru$f$ residue also attached at position 6 as in neokestose, and gives rise to 6-*O*-acetyl-1,5-anhydro-2,3,4-tri-*O*-methyl-D-glucitol (**4**). Here

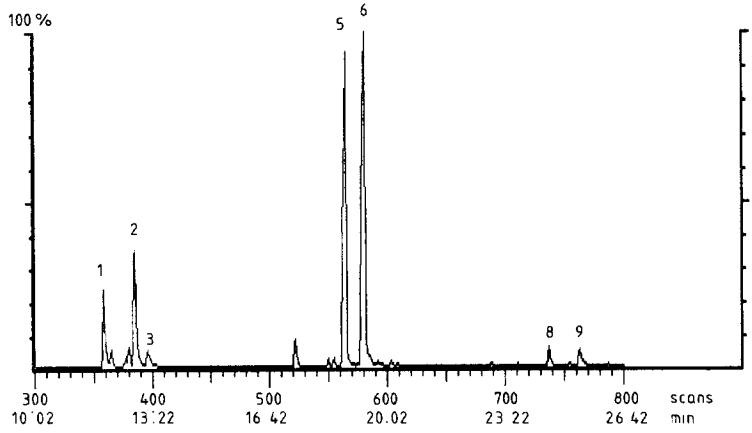


Fig. 2. GLC (see Experimental) of the products of the reductive cleavage of the fructan from *Pucinella peisonis*: **1–3**, **5**, **6**, **8**, and **9** as in Fig. 1.

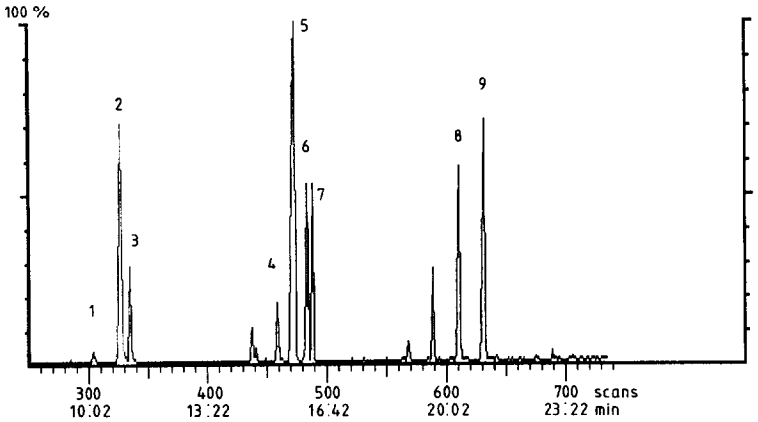


Fig. 3. GLC (see Experimental) of the products of the reductive cleavage of sinistrin: **1–3** and **5–9** as in Fig. 1; **4**, 6-*O*-acetyl-1,5-anhydro-2,3,4-tri-*O*-methyl-D-glucitol.

again, a peak was observed at 590 and calculated as for the quack-grass fructan. The mass spectrum of **4** was identical with that of the product derived from the 6-substituted α-D-Glc*p* unit in pullulan (Table I).

Table II shows the proportions of the several types of linked β-D-Fru*f* and α-D-Glc*p* residues in sinistrin, and the fructans from quack grass and *P. peisonis*.

^{13}C NMR spectroscopy confirmed the (2 → 1)-linked structure of inulin and the chemical shift data are the same as those reported[17–19]. The spectrum of the fructan from *P. peisonis* is similar to those in the literature for levans[18,20]. The (2 → 6)-linked structure and the low dp (~ 10) of this fructan was verified. The α-D-Glc*p* residue was linked only at the 1 position as in sucrose (C-1 resonance at δ 93.0). The fructan of quack grass gave a more complex spectrum with signals for C-2 at δ 105 and C-6 at δ 64.2 characteristic of (2 → 6)-linked β-D-Fru*f* residues, whereas the branched β-D-Fru*f* residues had resonances for C-2 at δ 104.3 and C-6 at δ 64.0[18–21].

The spectrum of sinistrin (Fig. 4) contained six groups of intense signals due to the fructosyl carbon atoms. There are at least 4 different signals for C-2 of β-D-Fru*f* residues; the signal at δ 104.0 is typical for C-2 of a (2 → 1)-linked residue, those at δ 104.5 and 104.7 are assigned to terminal and branched residues, respectively, and that at δ 104.88 to a (2 → 6)-linked residue. The occurrence of (2 → 6)-linked residues is also supported by the signal at δ 81.0, which can be attributed to C-5 of such a unit or a branched residue[20]. For residues not 6-substituted, the resonance for C-5 was at δ 81.9. Strong evidence for (2 → 6)-linked D-Fru*f* residues is the broad signal at δ 63.98, which is characteristic of 6-substitution. The signals of the α-D-Glc*p* residues were not detected due to the small proportion of α-D-Glc*p* in the molecule. Table III shows the NMR data of the fructans.

TABLE I

EI mass spectra (relative abundance in parentheses)

1,5-Anhydro-2,3,4,6-tetra-*O*-methyl-D-glucitol (**1**): *m/z* 41 (30%), 43 (45), 45 (100), 55 (12), 58 (18), 59 (21), 71 (52), 75 (37), 83 (12), 85 (16), 88 (24), 99 (13), 101 (63), 102 (10), 111 (12), 115 (10), 143 (18).

2,5-Anhydro-1,3,4,6-tetra-*O*-methyl-D-mannitol (**2**): *m/z* 41 (21%), 43 (17), 45 (100), 55 (10, 59 (15), 71 (46), 75 (19), 83 (10), 99 (13), 101 (41), 111 (31), 115 (19), 125 (26), 126 (11), 143 (28), 156 (10), 157 (8), 189 (6), 221 (16).

2,5-Anhydro-1,3,4,6-tetra-*O*-methyl-D-glucitol (**3**): *m/z* 41 (23%), 43 (16), 45 (100), 55 (10), 59 (13), 71 (29), 75 (10), 87 (11), 89 (9), 99 (10), 101 (53), 111 (23), 115 (10), 125 (5), 143 (18), 221 (6).

6-*O*-Acetyl-1,5-anhydro-2,3,4-tri-*O*-methyl-D-glucitol (**4**): *m/z* 41 (28%), 42 (19), 43 (100), 45 (48), 55 (10), 58 (20), 59 (17), 69 (12), 71 (30), 73 (18), 75 (44), 87 (56), 88 (30), 101 (30), 130 (11), 143 (6), 217 (6).

1-*O*-Acetyl-2,5-anhydro-3,4,6-tri-*O*-methyl-D-mannitol (**5**): *m/z* 41 (29%), 42 (16), 43 (100), 45 (81), 53 (12), 55 (14), 59 (13), 69 (12), 71 (72), 72 (12), 75 (17), 81 (15), 83 (19), 84 (10), 85 (20), 97 (11), 99 (11), 101 (49), 111 (52), 113 (11), 114 (15), 115 (27), 117 (18), 125 (31), 126 (27), 127 (16), 143 (34), 155 (7), 156 (6), 157 (18), 187 (6), 203 (6), 249 (30).

6-*O*-Acetyl-2,5-anhydro-1,3,4-tri-*O*-methyl-D-glucitol (**6**): *m/z* 41 (30%), 42 (16), 43 (100), 45 (77), 55 (11), 59 (14), 69 (12), 71 (42), 83 (11), 85 (12), 87 (16), 101 (45), 111 (35), 114 (14), 115 (15), 117 (29), 125 (10), 143 (16), 155 (7), 156 (6), 249 (8).

1-*O*-Acetyl-2,5-anhydro-3,4,6-tri-*O*-methyl-D-glucitol (**7**): *m/z* 41 (30%), 42 (16), 43 (100), 45 (77), 55 (12), 59 916), 69 (14), 71 (51), 73 (11), 75 (13), 81 (13), 83 (11), 84 (11), 85 (17), 87 (16), 99 (10), 101 (48), 111 (34), 114 (17), 115 (13), 117 (17), 125 (15), 126 (27), 143 (12), 157 (9), 158 (6), 175 (6), 187 (6), 217 (9), 249 (11).

1,6-Di*O*-acetyl-2,5-anhydro-3,4-di-*O*-methyl-D-mannitol (**8**): *m/z* 41 (16%), 42 (13), 43 (100), 45 (16), 71 (26), 83 (10), 87 (16), 101 (13), 111 (18), 117 (13), 124 (17), 143 (9), 157 (4), 171 (4), 185 (5), 216 (10).

1,6-Di-*O*-acetyl-2,5-anhydro-3,4-di-*O*-methyl-D-glucitol (**9**): *m/z* 41 (17%), 42 (11), 43 (100), 45 (18), 71 (26), 83 (10), 87 (13), 101 (17), 111 (15), 117 (21), 124 (16), 125 (10), 143 (4), 156 (6), 157 (6), 203 (6), 216 (11).

DISCUSSION

The results of the reductive cleavage and the NMR analysis indicated the β-D-fructan from quack grass to have a highly branched structure with a (2 → 6)-linked backbone and a small proportion of unbranched (2 → 1)-linked β-D-Fru*f* residues. These results are in good agreement with those of Hammer and Morgenlie[21]. The β-D-fructan of *Pucinella peisonis* had mainly a (2 → 6)-linked unbranched structure with a relatively low dp of ~ 10. The reductive cleavage gave 1,5-anhydro-2,3,4,6-tetra-*O*-methyl-D-glucitol as the only glucose-derived unit; thus, the α-D-Glc*p* residues were in terminal positions. This fructan has potential as a substrate for the determination of β-D-fructan (2 → 6) exohydrolases because of its mainly unbranched structure.

Sinistrin, isolated from red squill, had the most complex structure of the fructans investigated. It was highly branched and contained (2 → 1)- (33%) and (2 → 6)-linked (~ 19%), terminal (25%), and branched (19%) β-D-Fru*f* residues.

TABLE II

Percentages and numbers of β-D-Fru*f* and α-D-Glc*p* residues per molecule of fructan

		Sinistrin		Quack grass		*Pucinella peisonis*	
		Percentage	Number	Percentage	Number	Percentage	Number
β-D-Fru*f*	terminal	25.2 ± 0.8	7.9 ± 0.2	35.7 ± 1.1	16.3 ± 0.5	15.6 ± 0.5	1.7 ± 0.1
	1-linked	33.1 ± 3.1	10.4 ± 1.0	3.1 ± 3.1	1.4 ± 0.1		
	6-linked	19.2 ± 1.9	6.0 ± 0.6	22.4 ± 2.2	10.2 ± 1.0	72.5 ± 7.2	8.0 ± 0.8
	1,6-linked	19.4 ± 0.6	6.1 ± 0.2	36.5 ± 1.1	16.6 ± 0.5	5.3 ± 0.2	0.6 ± < 0.1
α-D-Glc*p*	terminal	0.54 ± 0.1	0.2 ± < 0.1	2.1 ± 0.1	1.0 ± < 0.1	6.6 ± 0.2	1.0 ± < 0.1
	6-linked	2.7 ± 0.1	0.8 ± < 0.1				
Average dp		31		45		10	
Ratio unbranched/branched		5.2		2.7		18.9	

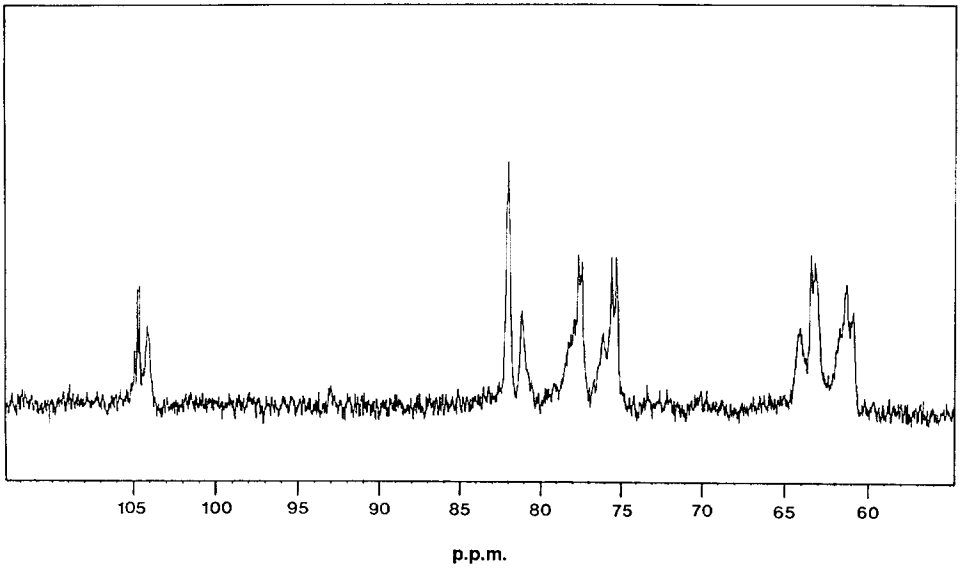


Fig. 4. Proton-decoupled ^{13}C NMR spectrum (75 MHz) of a solution of sinistrin in D_2O at 20°.

TABLE III

Assignments (δ) for the ^{13}C NMR spectra of the β-D-fructans

Inulin			Fructan from *Pucinella peisonis*		
	→ 1)-Fru*f*-(2 →		α-D-Glc*p*-(1 →	Fru*f*-(2 →	→ 6)-Fru*f*-(2 →
C-1	61.7		93.0	60.8	60.8
C-2	104.1		71.9	104.8	105.1
C-3	77.8		73.5	77.5	77.2
C-4	75.1		70.2	75.4	76.1
C-5	81.9		73.2	82.0	81.2
C-6	63.0		61.5	63.3	64.3

Sinistrin from red squill				
	→ 6)-Fru*f*-(2 →	→ 1)-Fru*f*-(2 →	Fru*f*-(2 →	→ 1,6)-Fru*f*-(2 →
C-1	60.7	61.2	60.8	60.8 [a]
C-2	104.9	104.0	104.5	104.6
C-3	77.3	77.5	77.5 [a]	77.5 [a]
C-4	76.0	75.2	75.4	75.9
C-5	81.0 [a]	81.9 [a]	81.9 [a]	81.0 [a]
C-6	64.0 [a]	63.1	63.4	64.0 [a]

Fructan from quack grass			
	(→ 6)-Fru*f*-(2 →	Fru*f*-(2 →	→ 1,6)-Fru*f*-(2 →
C-1	60.8	60.8	61.2
C-2	105.0	104.5	104.3
C-3	77.1	77.5	77.5
C-4	75.7	75.1	75.7
C-5	81.1	81.9	81.1
C-6	64.2	63.1	64.0

[a] Unresolved from other signals.

The reductive cleavage method, but not methylation analysis, can discriminate between (2 → 1)- and (2 → 6)-linked β-D-Fru*f* residues[14].

Of the 3.24% of the α-D-Glc*p* residues, 0.54% were linked as in sucrose and 2.7% as in neokestose. This type of linkage was found in fructans of the *Liliacea*[22] and the New Zealand cabbage tree[23]. The proportions of (2 → 6)-linked and branched β-D-Fru*f* residues were similar. Fig. 5 shows the suggested model for a sinistrin molecule with a dp of 12. The average dp was calculated to be 31, a value higher than those reported by Nitsch et al.[6] but in good agreement with the results of Eigner et al.[7]. Calculation of the number of the various fructose residues in a molecule of average dp of 31 gives 10.4 (2 → 1)-linked, 6 (2 → 6)-linked, 7.9 terminal, and 6.1 branched β-D-Fru*f*. Of the α-D-Glc*p* residues, 0.2 were (1 → 2)-linked and 0.8 were also 6-substituted by β-D-Fru*f*. On an average, the difference between terminal and branched β-D-Fru*f* moieties in the molecule is 1.8, which

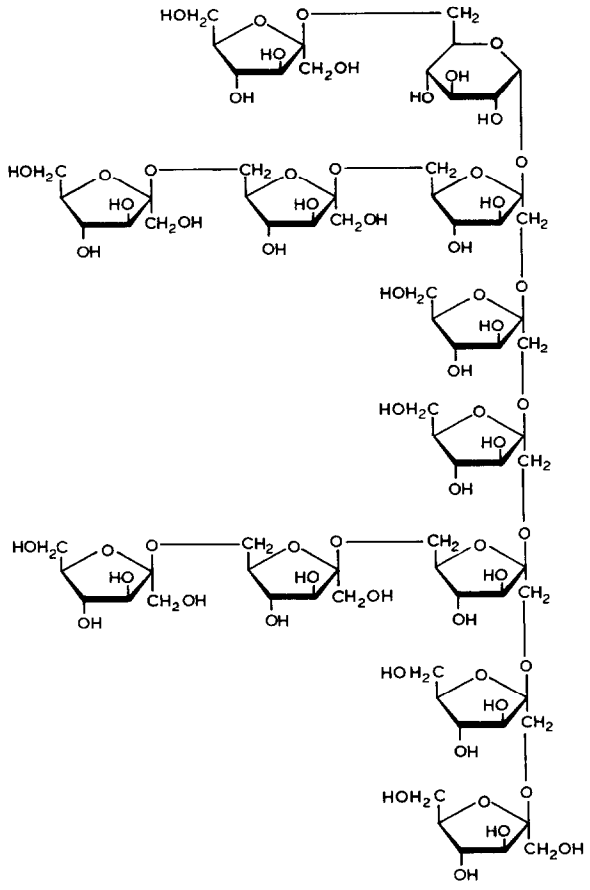


Fig. 5. One possible structure for sinistrin.

accords with the average number of the two types of α-D-Glc*p* residues. Hence, a model structure is proposed with a (2 → 1)-linked backbone with branches of (2 → 6)-linked β-D-Fru*f* residues. Each branch is terminated by a β-D-Fru*f* residue. This model is only one of several possible structures.

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