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Biochemistry

Structure of Glucofructan from Bulbs of *Galanthus platyphyllus* Traub et Moldenke (Amaryllidaceae)

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ABSTRACT. Hot water extraction of bulbs of *Galanthus platyphyllus* Traub et Moldenke followed by amylolysis (to remove starch), chromatography on DEAE-cellulose (to remove acidic arabinogalactan) and elimination of acetylated glucomannan by alkaline saponification (to remove water-insoluble deacetylated glucomannan) afforded glucofructan. According to ^{13}C NMR spectral data it belongs to the branched type (mixed-linkage type) fructans, containing both inulin and levan type structures. *© 2007 Bull. Georg. Natl. Acad. Sci.*

Key words: *glucofructan, polysaccharide, Galanthus platyphyllus.*

Several fructans were shown to exhibit pronounced activity against a series of different tumor types [1].

As part of our continued interest in the structure-activity relationship of glucofructans, a comparative structural elucidation of glucofructans of some plant species occurring in Georgia has been recently reported [2].

In the previous paper the structure investigation of acetylated glucomannan of *Galanthus platyphyllus* was carried out and, besides, the presence of glucofructan in crude polysaccharide preparation was shown as well [3].

The species of Amaryllidaceae family have been shown to contain glucofructans similar in chemical structure to those found in Liliaceae [4,5].

In the present paper the structure elucidation of glucofructan from *G. platyphyllus* bulbs is described.

Methods. Extraction of crude polysaccharides was carried out as described in [3,6].

Specific rotation was measured with a Jasco model DIP-360 digital polarimeter.

^{13}C Nuclear magnetic resonance(NMR) spectrum was recorded with a Bruker AM-300 spectrometer (75.43 MHz) using 2% solution of polysaccharide in deuterium oxide at 30^{o} (internal standard - methanol, 50.15 p.p.m. from tetramethylsilane). Fructose was determined by the resorcinol method [7], glucose - by the glucose oxidase method [8].

Treatment of polysaccharides with α-amylase, chromatography on DEAE-cellulose column and alkaline solution. 500 mg of crude polysaccharide preparation was treated by α-amylase [6], yield 275 mg (55%). 270 mg obtained product was loaded onto DEAE-cellulose column (3x30 cm) in carbonate form [6]. Elution was performed with water, yield 150 mg (55.6%). 130 mg obtained preparation was treated with 30 ml 0.1 M NaOH at room temperature for 3 hours. Then the solution was neutralized with 0.1 N CH_3COOH. The residue was removed by centrifugation [9]. The supernatant was dialyzed against distilled water, evaporated, yield 60 mg (46.2%,). Composition: fructose (FRU) - 85.8 %, glucose (GLC) - 2.2 %.

Results and discussion. The glucofructan was shown to be one of the main water-soluble polysaccharides of *G. platyphyllus*. It was isolated by hot water extraction and purified from starch by amylolysis, from acidic arabinogalactan by chromatography on DEAE-cellulose column and from acetylated glucomannan by alkaline

Table 1

Characteristics of *G. platyphyllus* glucofructan

Yield*, %	$[\alpha]_D$, (c 0.3, H_2O)	FRU, %	GLC, %	Ratio FRU:GLC
4.14	- 35°	85.8	2.2	39:1

* Based on dry biomass

saponification, when due to deacetylation and precipitation of water-insoluble deacetylated glucomannan pure glucofructan preparation was obtained. The charasteristics of *G. platyphyllus* glucofructan are given in Table 1.

The specific optical rotation of fructan is in the range observed for polysaccharides that contain mainly β-D-fructofuranose residues. The ease with which the polysaccharide underwent hydrolysis (0.05M trifluoroacetic acid, 45 min., at 100°C) suggested that the D-fructose residues were present in the furanose form. The average degree of polymerization is 40 monosaccharide residues.

The glucofructan gave a single peak on gel chromatography with molselect G-25, G-50 and G-75. The symmetrical elution profile of polysaccharide, obtained by gel filtration, indicated its homogeneity.

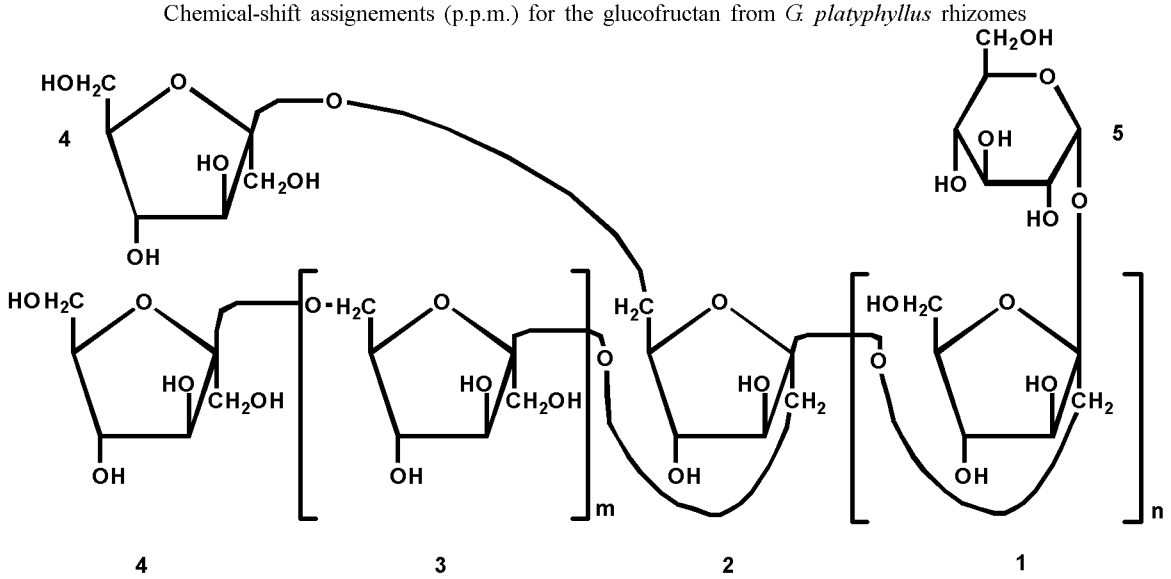
The structure of the glucofructan was studied by means of ^{13}C NMR spectroscopy. The interpretation of the ^{13}C NMR spectrum was made in accordance with [10] (Table 2).

The spectrum is similar to the ^{13}C NMR spectra of glucofructans of *Symphytum asperum*, *S.caucasicum* (Boraginaceae) [10] and *Muscari szovitsianum*, *Ornithogalum ponticum* [11, 12] and *Polygonatum glaberrimum* (Liliaceae) [13]. Thus, the glucofructan of *G. platyphyllus*, by analogy with glucofructans from the above-mentioned other plants, is branched and built of 2→1 and 2→6-linked β-D-fructofuranose residues. 1,2,6-Trisubstituted residues of β-D-fructofuranose serve as branching points of the polymeric molecules. Like many other fructans of higher plants, the glucofructan from *G. platyphyllus* was shown to contain α-D-glucopyranose residues as terminal non-reducing groups linked 1→2 to β-D-fructofuranose residues.

Thus, glucofructan of *G. platyphyllus* belongs to the branched type (mixed-linkage type) fructans, having both inulin and levan type structures.

Table 2

Chemical-shift assignements (p.p.m.) for the glucofructan from *G. platyphyllus* rhizomes



Residue	C1	C2	C3	C4	C5	C6
β-D-fructosyl						
1	62.7	104.4	78.3	75.8	82.24	63.5
2	61.9	104.6	77.9	76.2	81.5	63.8
3	61.6	105.1	77.9	76.5	81.2	64.4
4	61.6	104.9	77.9	75.3	82.5	63.5
α-D-glucosyl						
5	93.6	72.5	74.0	70.6	73.7	61.3

ბიოქიმია

ბრტყელფოთოლა თეთრყვავილას *Galanthus platyphyllus* Traub et Moldenke (Amaryllidaceae) ბოლქვების გლუკოფრუქტანის სტრუქტურა

ვ. ბარბაქაძე, კ. მულკიჯანიანი, მ. მერლანი, ლ. გოგილაშვილი, ლ. ამირანაშვილი

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(წარმოდგენილია აკადემიის წევრის ნ. ნუცუბიძის მიერ)

ნაჩვენებია, რომ გლუკოფრუქტანი წარმოადგენს *Galanthus platyphyllus*-ის ფესურების ერთ-ერთ ძირითად წყალში ხსნად პოლისაქარიდს. ^{13}C ბირთვულ-მაგნიტური რეზონანსის (ბმრ) სპექტროსკოპიის მონაცემების საფუძველზე ის მიეკუთვნება განტოტვილი ტიპის (შერეული ბმების შემცველ) ფრუქტანებს, რომლებიც ერთდროულად შეიცავენ როგორც ინულინის, ისე ლევანის ტიპის სტრუქტურებს.

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