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GLUCOFRUCTANS FROM *Taraxacum officinale* ROOTS

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The structure of glucofructans from Taraxacum officinale *roots growing in Buryatia was studied by chemical, chromatographic, and spectral methods. It was found that fructose,glucose, saccharose, 1-kestose, and nystose were present in the free state. The structures of the two dominant polymeric compounds, TGf-1 (5.7 kDa) and TGf-2 (2.6 kDa), which were linear inulin-type macromolecules consisting of fructofuranose units bonded through β-(2→1)-bonds, were studied.*

Key words: *Taraxacum officinale*, Asteraceae, glucofructans.

Taraxacum officinale Wigg. (Asteraceae) is an official medicinal plant that together with *Inula helenium* and *Arctium* spp. provides raw material containing inulin-type glucofructans [1-3]. A total of 22 species of the *Taraxacum* Wigg. genus have been observed in the flora of Buryatia. Reserves of the pharmacopoeic species *T. officinale* are sufficient to enable its industrial production within the Republic [4]. The goal of our work was to study the composition and structure of glucofructans from *T. officinale* roots growing in the Republic of Buryatia.

Polysaccharides were isolated by treating plant material beforehand with a series of solvents (hexane, $CHCl_3$, ethylacetate) to remove lipophilic and phenolic compounds, after which carbohydrates were extracted from the raw material by ethanol (80%). The components soluble in alcohol were identified by chromatography (HPTLC) as fructose, traces of glucose, and several oligosaccharides, from which saccharose, 1-kestose, and nystose were isolated and identified. The content of free carbohydrates (calculated as fructose) in *T. officinale* roots was 15.35% of the air-dried mass of raw material (13.16-17.95% in commercial raw material).

Water-soluble polysaccharides (TOW, 85.65% fructose content) were isolated from the aqueous extract. Gel filtration of them produced a TOW′ component containing 92.31% fructose. Gel chromatography over Sephadex G-75 indicated that TOW′ was heterogeneous. Therefore, fractionation by step-wise precipitation with ethanol was carried out to produce more homogeneous fractions. A total of six fractions was obtained (Table 1).

All fractions contained fructose and glucose in various proportions. The molecular weights of the fraction components were in the range 2.0-10.4 kDa. Further studies were carried out with the dominant fractions TOW′-2 and TOW′-5, designated TGf-1 and TGf-2. Table 2 lists their main characteristics.

It was found that TGf-1 and TGf-2 were homogeneous with molecular weights 5.7 and 2.6 kDa, respectively. The negative specific rotation was consistent with the presence of the β-configuration of the glycoside bonds in the polymer macromolecules. According to HPTLC, GC—MS, and ^{13}C NMR spectra, the glucose:fructose ratios were 1:33.4-34.6 and 1:13.3-13.9 for TGf-1 and TGf-2. IR spectra of the studied compounds exhibited absorption bands characteristic of inulin-type glucofructans (Table 2).

Periodate oxidation of TGf-1 and TGf-2 was complete in 110-115 h. The periodate consumption was 0.98 and 0.99 mol/anhydro unit; HCOOH release, 0.03 and 0.07 mol/anhydro unit, respectively. Smith degradation products contained only glycerine, which was consistent with (2→1)-bonds between hexose units in the polymer chains.

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TABLE 1. Characteristics of Fractions TOW′

Fraction	C_{EtOH}, %	Yield, %	$[\alpha]_D^{20}$, ° (*c* 3.0, H_2O)	Frc**	Glc**	MW, kDa
TOW′-1	45	1.15	-	+	Tr.	10.4–6.1
TOW′-2	67	58.80	–39.6	97.1	2.8	5.7
TOW′-3	73	6.63	–33.3	95.2	4.7	3.5–5.6
TOW′-4	77	5.30	–31.6	94.7	5.2	2.6–3.5
TOW′-5	90	14.13	–27.4	93.2	6.7	2.6
TOW′-6	95	8.03	-	+	+	2.0–1.5

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*of TOW′ mass; **mol%.

TABLE 2. Characteristics of Glucofurctans TGf-1 and TGf-2

Glucofructan	Glc–Frc ratio from data of			Periodate oxidation, mol		IR spectroscopy (ν, cm^{-1})	Ratio of methylated carbohydrates		
	HPTLC	GC–MS	^{13}C NMR	$NaIO_4$	HCOOH		3,4,6-tri-*O*-Me-Frc*p*	1,3,4,6-tetra-*O*-Me-Frc*p*	2,3,4,6-tetra-*O*-Me-Glc*p*
TGf-1	1:34.6	1:33.4	1:33.7	0.98	0.03	3375, 2945, 1425, 1330, 1254, 1246, 1138, 1090, 1073, 1032, 916, 872, 848, 820	94.2	2.8	2.9
TGf-2	1:13.9	1:13.3	1:13.8	0.99	0.07	3384, 2957, 1421, 1330, 1255, 1240, 1143, 1085, 1070, 1034, 915, 869, 850, 819	86.2	6.7	7.0

TABLE 3. ^{13}C NMR Data for Glucofructans TGf-1 and TGf-2

Glucofructan	Group	^{13}C chemical shifts, ppm					
		C-1	C-2	C-3	C-4	C-5	C-6
TGf-1	-2-β-D-Frc*f*-1-	61.04	103.85	77.16	75.16	82.32	62.82
	1-α-D-Glc*p*-	94.03	71.33	72.92	70.02	72.12	60.35
TGf-2	-2-β-D-Frc*f*-1-	61.59	103.89	78.40	75.30	82.40	62.93
	1-α-D-Glc*p*-	93.48	71.12	73.39	70.01	72.09	60.42

Methylation by the Ciucanu-Capita method, formolysis, and hydrolysis of the permethylates followed by chromatography (HPTLC, GC—MS) detected 3,4,6-tri-*O*-Me-Frc*p*, 1,3,4,6-tetra-*O*-Me-Frc*p*, and 2,3,4,6-tetra-*O*-Me-Glc*p* in 33:1:1 (TGf-1) and 12:1:1 (TGf-2) ratios. The detection of 3,4,6-tri-*O*-Me-Frc*p* as the principal methylation product and the absence of disubstituted fructose in the hydrolysate indicated that both polymers consisted of (2→1)-bonded fructose units and were linear macromolecules.

Thus, glucofructans from *T. officinale* roots were linear inulin-type polymers consisting of β-(2→1)-bonded fructofuranose and had the following structures:

TGf-1: Glc*p*-1-(2-Frc*f*-1)$_{32}$-2-Frc*f*

TGf-2: Glc*p*-1-(2-Frc*f*-1)$_{12}$-2-Frc*f*.

EXPERIMENTAL

Roots of *T. officinale* were collected near Lake Baikal in the Republic of Buryatia in September 2007 (Russia). Fructose (CAS No. 57-48-7, Acros Organics), glucose (CAS No. 50-99-7, Acros Organics), inulin (CAS No. 9005-80-5, Acros Organics), 1-kestose (CAS No. 470-69-9, Carbosynth Lim.), and nystose (CAS No. 13133-07-8, Carbosynth Lim.) were used as standards.

HPTLC was carried out on Sorbfil PTSKh-AF-V plates (Sorbpolimer). The solvent systems were *i*-PrOH:$CHCl_3$:H_2O (7:4:1, 1), double elution at 4 and 8 cm; *i*-PrOH:H_2O (4:1, 2), benzene:EtOAc (2:1, 3), and BuOH:Py:H_2O (15:30:20, 4). Developers were urea:H_3PO_4 (1), *p*-diphenylamine:aniline:H_3PO_4 (2), and $KMnO_4$:$NaIO_4$:benzidine (3).

Optical rotation was determined on a SM-3 polarimeter (Zagorsk Optico-Mechanical Plant) in a 1-dm cuvette at 20°C. IR spectra in film on KRS-5 plates were recorded in the range 4,000-650 cm^{-1} on a Spectrum 100 (Perkin—Elmer) IR-Fourier spectrometer. ^{13}C NMR spectra were recorded on a VXR 500S (Varian) NMR spectrometer at operating frequency 125.7 MHz. Spectra were recorded in DMSO-d_6 solutions (1%). GC—MS analysis was performed in an Agilent GC—MS with a mass-selective detector (No. 5973) with a diffusion pump using a PH-Innowax capillary column (30 m × 250 μm, 0.50 μm). The temperature gradient was 150-250°C; heating rate, 2°C/min; carrier gas, He; flow rate, 1 mL/min.

Isolation of Water-soluble Polysaccharides. Ground raw material (125 g) was extracted in a Soxhlet apparatus successively with hexane, $CHCl_3$, and EtOAc. Solvents were removed. Raw material was extracted three times with ethanol (80%) at 100°C. The combined extract was concentrated in vacuo to an aqueous residue that was dried in a vacuum chamber to afford a fraction (12.54 g) of carbohydrates soluble in alcohol (10.03% of the raw material mass) that was analyzed by HPTLC (system 1, developer 1).

The raw material remaining after ethanol extraction was extracted with water (1:25 ratio) at 100°C for 1 h. The extract was filtered. The extraction was repeated three times. The combined filtrate was concentrated in vacuo to 100 mL and precipitated by ethanol (95%). The resulting precipitate (TOW) was centrifuged and dried. Yield of TOW, 22.415 g (17.93% of the raw material mass). The fructose content (c_{Frc}) in TOW (resorcinol method [5]) was 85.65 ± 2.19%.

The contents of free carbohydrates (FC) and glucofructans (GFr) in *T. officinale* raw material were determined by the resorcinol method. The observed FC/GFr ratios (%) were 15.35/28.39 in raw material; ZAO Zdorov′e, 15.05/15.27; OOO Start-Fito, 17.95/33.05; OOO AlekC+, 13.16/33.42.

Isolation of Oligosaccharides. The fraction of carbohydrates soluble in alcohol was separated by preparative PC (FN-2 paper, Filtrak) in descending mode (system 2). Bands of oligosaccharides were cut out and eluted with water. The eluates were concentrated and rechromatographed using HPTLC (system 1) to afford compounds TO-1, TO-2, and TO-3, which were identified based on optical rotation, monosaccharide composition, and methylation with saccharose { $[\alpha]_D^{20}$ +65° (*c* 2.0, H_2O); Frc:Glc 1:1; 1,3,4,6-tetra-*O*-Me-Frc*p*:2,3,4,6-tetra-*O*-Me-Glc*p* 1:1), 1-kestose { $[\alpha]_D^{20}$ +25° (*c* 1.1, H_2O); Frc:Glc 2:1; 3,4,6-tri-*O*-Me-Frc*p*:1,3,4,6-tetra-*O*-Me-Frc*p*:2,3,4,6-tetra-*O*-Me-Glc*p* 1:1:1), and nystose { $[\alpha]_D^{20}$ +15° (*c* 1.2, H_2O); Frc:Glc 3:1; 3,4,6-tri-*O*-Me-Frc*p*:1,3,4,6-tetra-*O*-Me-Frc*p*:2,3,4,6-tetra-*O*-Me-Glc*p* 2:1:1).

Gel-filtration over Molselect G-25. TOW (10 g) was dissolved in water (150 mL). The resulting solution was transferred to a column packed with Molselect G-25 (3 × 90 cm) and eluted with water. Fractions (50 mL) were collected and analyzed for carbohydrates (phenol—H_2SO_4). Fractions containing carbohydrates were combined and precipitated with acetone. The resulting precipitates were centrifuged and dried to produce component TOW′ (6.50 g, 65% of the TOW mass) with fructose content 92.31 ± 1.84%.

Fractionation by Stepwise Precipitation with Ethanol. TOW′ (5 g) was dissolved in water (100 mL) and treated in portions with ethanol (95%). The resulting precipitates were centrifuged and dried to afford six fractions TOW′-1 (57 mg), TOW′-2 (2.940 g), TOW′-3 (0.331 g), TOW′-4 (0.265 g), TOW′-5 (0.706 g), and TOW′-6 (0.401 g).

Total hydrolysis of polysaccharides was performed in H_2SO_4 (1 M) at 80°C for 30 min, after which the hydrolysate was treated with anion-exchanger AV-17-8 (HCO_3^--form), concentrated to the minimal volume in vacuo at 40°C, and analyzed by HPTLC (system 1, developer 1). The quantative monosaccharide composition was determined by a densitometric method [6].

Gel-chromatography was performed over Sephadex G-50 (2.5 × 80 cm, Pharmacia, Uppsala) with elution by water at 0.1 mL/min and detection of elutions by the phenol—H_2SO_4 method. The standards were dextrans of molecular weight 2,000, 80, 50, and 10 kDa (all Pharmacia).

Methylation was carried out by the Ciucanu—Capita method [7], for which polysaccharide (30 mg) was dissolved in water (100 μL), diluted with DMSO (4.5 mL), stirred for 20 min, treated with finely ground NaOH (120 mg) and with another portion of NaOH (350 mg) after 10 min, and stirred until a homogeneous suspension formed. The mixture was treated with MeI (360 μL), stirred at room temperature for 10 min, treated with water (6 mL) and with ethanol (95%, 100 mL) after completely dissolved. The resulting precipitate was separated by centrifugation. The methylation was repeated another two times. After the third procedure, the methylation product was extracted by CH_2Cl_2. The solvent was removed. The product

was analyzed by IR spectroscopy. The methylation was repeated until the absorption band of the hydroxyl groups disappeared in the IR spectrum.

Formolysis and Hydrolysis of the Permethylate. The permethylate (15 mg) was treated with formic acid (5 mL, 90%) and heated at 100°C for 1 h. Formic acid was removed in vacuo in the presence of methanol. The dry residue was dissolved in H_2SO_4 (3 mL, 1 M) and heated at 100°C for 24 h. The hydrolysate was studied by HPTLC (system 3, developer 2) and GC—MS. The hydrolysates of permethylates TGf-1 and TGf-2 contained 3,4,6-tri-*O*-Me-Frc*p*, 1,3,4,6-tetra-*O*-Me-Frc*p*, and 2,3,4,6-tetra-*O*-Me-Glc*p*.

Periodate Oxidation and Smith Degradation. The studied polymer (50 mg) was dissolved in water (30 mL), treated with $NaIO_4$ solution (10 mL, 0.2 M), and left at 4°C. An aliquot of the solution was taken every 24 h and analyzed for IO_4^- content (spectrophotometric method based on the absorption decrease at 223 nm [6]) and HCOOH [titration with NaOH solution (0.01 M)]. The reaction was stopped after 10 d by adding ethyleneglycol (1 mL). Water (20 mL) and $NaBH_4$ (200 mg) were added. The solution was treated after 12 h with KU-2-8 cation-exchanger (30 g, H^+-form). The filtrate was concentrated to dryness. The dry solid was dissolved in H_2SO_4 (5 mL, 1 M) and heated at 100°C for 6 h. The hydrolysate was treated with anion-exachanger AV-17-8 (HCO_3^--form), concentrated to the minimal volume in vacuo at 40°C, and analyzed by HPTLC (system 4, developer 3).

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