GLUCOFRUCTANS FROM *Arctium tomentosum* ROOTS

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Roots of Arctium tomentosum *(Compositae) were found to contain water-soluble polysaccharides (glucofructans), pectinic substances, and hemicellulose. The structure of the glucofructan was established by chemical methods and IR and ^{13}C NMR spectroscopies.*

Key words: *Arctium tomentosum*, fractional composition, water-soluble polysaccharides, pectinic substances, hemicellulose, glucofructan, structure.

Most plants of the Compositae family can accumulate radioactive substances and microelements [1] and produce significant quantities of ecdysones, thereby posing interesting problems and solutions [2].

Arctium tomentosum [3] is a weedy perennial that reaches heights of 100-200 cm and is one of the principal carbohydrate-bearing plants [4] distributed widely in Kyrgyzstan. It grows in dense thickets on mountains and in pastures and hay fields.

Herein we present our research results on the composition of glucofructans isolated from *A. tomentosum* roots collected during fruiting in Strel′nikov village in the Chui valley of the Kyrgyz Republic.

Dried and ground raw material was soaked in ethanol (96° and then 82°) to inactivate enzymes. The alcohol extracts were combined to produce mono- and oligosaccharides in 7.5% yield [5]. The remaining raw material was used to isolate water-soluble polysaccharides (WSPS), 24% [6]; pectinic substances (PS), 5.5%; and hemicellulose (HC), 7.4% [7]. The results showed that WSPS dominate in the plant roots.

WSPS are a slightly yellow amorphous powder that is very soluble in water at 60°C. The ash content after reprecipitation was 1.5%. Total acid hydrolysis of WSPS produced glucose and fructose according to paper chromatography (PC). Therefore, the WSPS are glucofructans (GF).

Gel chromatography of GF on Sephadex G-75 showed that they are heterogeneous [8, 9]. The average molecular weight (MW) varied in the range 10,000-16,000. The GF was fractionated from aqueous solution by alcohol to produce homogeneous fractions. Six fractions were obtained (Table 1).

Fractions 2, 3, and 4 differed in yield and MW whereas the specific rotation and fructose content were similar (Table 1). PC of the hydrolysates detected mainly fructose and traces of glucose. Therefore, fractions 2, 3, and 4 are glucofructans and were designated by us as GF-A, GF-B, and GF-C, respectively.

The IR spectra of GF-A, -B, and -C contain absorption bands (cm^{-1}) at 820 (pyranose ring), 860 (β-glycoside bond), and 940 (furanose ring).

The negative specific rotation angle and the IR spectra are consistent with the β-configuration of the glycoside bonds between the monosaccharides and the D-configuration of the fructose [10].

The GF were studied by periodate oxidation and methylation in order to determine the bond types and sizes of the oxygenated rings of the monosaccharides.

Periodate oxidation [11] of GF-A, -B, and -C found that consumption of oxidant remains constant after 120 h at 0.96, 0.98, and 0.98 mole per anhydro unit and 0.043, 0.042, and 0.042 moles of formic acid were formed. The products of Smith degradation [12] contained glycerine, the formation of which is consistent with the presence of β-(2→1) and β-(2→6) bonds.

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TABLE 1. Properties of Glucofructan Fractions

Fraction No.	Yield, %	$[\alpha]^{22}_D$ (*c* 1, water)	Fructose content, %	MW
1	0.5	-	-	-
A 2	20.5	-39.0	98.0	16600
B 3	47.0	-39.5	96.0	12000
C 4	24.0	-38.5	95.0	10000
5	3.0	-	-	-
6	2.0	-	-	-

TABLE 2. Properties of Permethylates of Glucofructans A, B, and C

Glucofructan	Yield, %	$[\alpha]^{22}_D$ (*c* 1.0, $CHCl_3$)	Ratio of sugars	
			3,4,6-tri-O-Me-D-Fru*f*	1,3,4-tri-O-Me-D-Fru*f*
A	84.0	-49	15	-
B	82.5	-50.5	14	-
C	82.2	-51.5	8	4

Ratio of 2,3,4,6-tetra-O-Me-D-Glu*p* to 3,4,6-tri-O-Me-D-Fru*f* = 1:2.

TABLE 3. Chemical Shifts (ppm) of C Atoms in ^{13}C NMR Spectra of *Arctium tomentosum* Glucofructans

Glucofructan	Group	C-1	C-2	C-3	C-4	C-5	C-6
A	-2-β-D-Fru*f*-1-	62.02	104.37	78.36	75.52	82.33	63.44
B	-2-β-D-Fru*f*-1-	61.97	104.46	78.15	75.47	82.01	63.42
C	-2-β-D-Fru*f*-1-	62.0	104.75	78.40	75.92	82.94	63.07
	-2-β-D-Fru*f*-16	62.20	105.05	78.68	76.59	82.40	63.89

Methylation of GF-A, -B, and -C by the Hakomori method [13] produced the permethylates, which were successively formylyzed and hydrolyzed by acid. The methylated sugars were identified by TLC. Their quantitative ratios were determined by GC (Table 2).

Analysis of the methylation products showed that the polymeric chain of GF-A, -B, and -C contains β-(2→1)-bonded fructofuranose units. This is confirmed by the principal product, 3,4,6-tri-O-Me-D-Fru (15, 14, and 8 parts). Besides the sugars mentioned above, GF-C also contains 4 parts 1,3,4-tri-O-Me-Fru, characteristic of β-(2→6)-bonds. These data were confirmed by ^{13}C-NMR studies of GF-A, -B, and -C (Table 3). It can be seen that the chemical shifts for GF-A, -B, and -C correspond to β-(2→1)-bonds. Signals for C-2 are observed in the spectra at 104.37, 104.46, and 104.75 ppm, which are assigned to fructofuranose units. Signals for C-4 appear at 75.52, 75.47, and 75.92 ppm. Additional signals at 105.05 and 76.59 ppm in the spectrum of GF-C belong to C-2 and C-4 in β-(2→6) glycoside bonds. The ratio of β-(2→1) to β-(2→6) bonds calculated by intensity integration was 1.5:1.0. The spectrum also contains a signal at 76.5 ppm that is typical of a C-4-bonded unit. GF of *Allium sativum* contain such mixed β-(2→1) and β-(2→6) bonds with branching at C-4 [14].

Thus, chemical and spectral data established that GF from *A. tomentosum* roots are biopolymers. GF-A and -B consist of β-(2→1)-bonded fructofuranose units of the inulin type. GF-C typically forms two types of polymeric chains: inulin β-(2→1) and levan β-(2→6).

The GF differ in fructose content and MW and have the following structures:

A: Glc*p*-1-[2Fru*f*1-]$_{92}$-2Fru*f*

B: Glc*p*-1-[2Fru*f*1-]$_{66}$-2Fru*f*

C: Glc*p*-1-[2Fru*f*1]$_{33}$ → [2Fru*f*6]$_{22}$ → 2Fru*f*

EXPERIMENTAL

Solutions were evaporated in a rotary evaporator at 40 ± 5°C. PC was performed on FN-9 and -11 paper by a descending method using *n*-butanol:pyridine:water (**1**, 6:4:3). TLC was carried out on Silufol UV-254 plates and on silica gel using $CHCl_3$:CH_3OH (**2**, 9:1) and benzene:acetone (**3**, 2:1). Spots were developed using anilinium acid phthalate (**1**, 10 min at 105°C) and periodate:$KMnO_4$:benzidine (**2**).

Specific rotation was measured on an SU-3 saccharimeter in a 1-dm tube of 10-mL volume at 20-23°C. Fructose was determined by the Kolthoff method [15].

IR spectra were recorded on a UR-20 instrument in KBr disks.

GC analysis was performed on a Tsvet-101 instrument with a flame-ionization detector under the following conditions: stainless-steel column (200 × 0.3 cm) packed with poly-1,4-butanediolsuccinamide (20%) on Chromaton NAW-CMCS (0.160-0.200 mesh), 190°C, He carrier gas (60 mL/min).

^{13}C NMR spectra were recorded on a Bruker WM-250 instrument at working frequency 62.89 MHz for C in 3% solutions of GF in D_2O with CH_3OH internal standard (50.15 ppm).

Raw Material Inactivation. Air-dried ground raw material (roots, 200 g) was inactivated in ethanol (600 mL, 96°) for 1 h and filtered. The remaining raw material was again extracted with ethanol (400 mL, 82°) for 30 min. The alcohol extracts were combined and evaporated to dryness. Yield of mono- and oligosaccharides, 15 g.

WSPS Isolation. Raw material after alcohol treatment and drying was extracted twice with water (1:8) at 75°C for 45 min. The extracts were combined, evaporated to half the volume, and treated with two volumes of ethanol. The resulting precipitate was filtered off; dehydrated; washed with alcohol (96°), acetone, and ether; and dried over P_2O_5. Yield, 48 g.

Polysaccharide Hydrolysis. Polysaccharide (0.05 g) in HCl (5 mL, 0.5%) was heated at 90°C for 45 min. The hydrolysate was neutralized with $BaCO_3$. The filtrate was concentrated to a syrup. PC (system **1**, developer **1**) detected mainly fructose and traces of glucose.

WSPS Fractionation. A solution of polysaccharides (100 mL, 5%) was treated with ethanol (100 mL) with vigorous stirring at ratios 1:1.5:2.0:2.5:3.0:3.5. Each fraction was centrifuged, washed with alcohol (96°) and acetone, and dried in vacuum over P_2O_5. Yields: 0.025, 1.025, 2.35, 1.20, 0.15, and 0.125 g (Table 1).

Gel Chromatography of Fractions 2, 3, and 4. These fractions (0.02 g each) were dissolved in distilled water (2 mL) and placed on a column (2.5 × 65 cm) of Sephadex G-75 (each chromatographed separately).

The column was calibrated by passage of dextrans of different MW (Dextran Standart 10000, 20000, 40000 Bio Chemica for GPC, Fluka). The eluate was collected (2-mL fractions) and analyzed by the phenol—H_2SO_4 method. The MW of the fractions was 16600, 12000, and 10000.

Periodate Oxidation and Smith Degradation. GF-A, -B, and -C (0.2 g each) were dissolved in water (50 mL), treated with sodium periodate solution (10 mL, 0.25 M), and left at 10°C in the dark. Aliquots (1 mL) were collected each day and titrated with sodium thiosulfate solution (0.01 N). The oxidation was finished in five days. The consumption of periodate was 0.98, 0.97, and 0.98 moles. The yield of formic acid was 0.047, 0.045, and 0.042 mole, respectively. The excess of periodate was destroyed by adding ethyleneglycol (1 mL). The oxidation product was reduced by an excess of $NaBH_4$ and hydrolyzed by H_2SO_4 (5 mL, 0.5 N) for 6 h at 100°C. PC of the hydrolysate (system **1**, developer **2**) detected glycerine.

Methylation of GF. GF-A, -B, and -C (0.05 g each) were methylated twice by the Hakomori method. The resulting product was extracted by $CHCl_3$ and evaporated to dryness. The yields were 0.042, 0.041, and 0.041, respectively (Table 2). The completeness of methylation was checked by TLC.

Formolysis and Hydrolysis of GF-A, -B, and -C Permethylates. Permethylates were heated on a boiling-water bath in formic acid (5 mL, 90%) for 1 h, cooled, and evaporated. The solid was dissolved in H_2SO_4 (2.5 mL, 0.5 N) and hydrolyzed at 100°C for 5 h. The hydrolysates were worked up as usual. The products were analyzed by TLC (systems **2** and **3**, developer **1**) and GC with authentic specimens (Table 2).

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