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GLUCOFRUCTANS FROM *Saussurea lappa* ROOTS

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Glucofructans from Saussurea lappa *(Asteraceae) roots were studied. It was found that free fructose and oligomeric glucofructans (saccharose, 1-kestose, nystose, 1^F-β-fructofuranosylnystose, and 1^F-β-fructofuranosyl-1^F-β-fructofuranosylnystose) were present. The dominant polymer Sl-GF (MW 51.4 kDa), which was a linear inulin-type glucofructan consisting of β-(2→1)-bonded fructofuranose units, was isolated and characterized. The total content of glucofructans in* Saussurea lappa *roots was 476.97–578.27 mg/g.*

Keywords: *Saussurea lappa*, Asteraceae, glucofructans.

Saussurea lappa C. B. Clarke [*S. costus* (Falc.) Lipsch.] is a member of the Asteraceae family and is widely used as a medicinal agent in China, India, and Tibet [1]. Roots of *S. lappa* (muxiang, yunmuxiang) are used in traditional Chinese medicine to treat headache, loss of appetite, diarrhea, and abdominal pain [2]. *S. lappa* (ru rta) is used in Tibetan medical practice for stomach ulcers and lung diseases and as an antihelminthic and hemostatic agent [3]. A study of the chemical composition of the roots of this plant showed that sesquiterpenes [4–7], triterpenes [5–9], lignans [10], phenylpropanoids [10, 11], chromones [9], simple phenols and phenolic acids [9, 10], flavonoids [12], anthraquinones [11], 2-pyrrolidinon-5(s)-carboxylate, 5-hydroxymethylfurfural, succinic acid, glucose [9], and essential oil [13] in addition to inulin were present. However, the chemistry of glucofructans (GFr) from this plant species has not previously been studied [14, 15]. The goal of our work was to study the composition and chemical structure of GFr from *S. lappa* roots.

GFr were isolated as follows. Ground roots of *S. lappa* were treated beforehand with several solvents to remove lipophilic and phenolic compounds. The remaining raw material was extracted with EtOH (80%) and H_2O to afford two fractions of alcohol- (SlF-1) and water-soluble GFr (SlF-2) in yields of 18.34 and 34.72% of the air-dried mass of raw material, respectively. The fructose contents in SlF-1 and SlF-2 were 67.53 ± 0.74 and 90.33 ± 1.08%, respectively.

A chromatographic study (HPTLC) of fraction SlF-1 showed that it contained fructose and a series of oligomeric GFr. The latter were identified by separating SlF-1 using ion-exchange and gel-permeation chromatography. As a result, five compounds were identified using methylation as saccharose (**1**), 1-kestose (**2**), nystose (**3**), 1^F-β-fructofuranosylnystose (**4**), and 1^F-β-fructofuranosyl-1^F-β-fructofuranosylnystose (**5**). All compounds were typical of GFr-containing plant raw material. However, they were observed in *S. lappa* for the first time. Quantitative analysis using an HPTLC-densitometric method showed that the fructose content in *S. lappa* roots was (mg/g) 22.91–50.15; in **1**, 13.14–47.04; **2**, 11.43–34.51; **3**, 2.21–15.68; **4**, 8.36–50.05; and **5**, 6.76–22.39 (Table 1).

The high fructose content and the IR spectrum of SlF-2 suggested that it contained polymeric GFr. Gel chromatography showed that SlF-2 was heterogeneous and contained compounds with molecular weights (MWs) 2.5-70 kDa. Ion-exchange chromatography over DEAE-cellulose and gel chromatographic separation over Molselect G-25 and Sephacryl S-300 HR were used to isolate the dominant polymer. As a result, homogeneous polymer Sl-GF was isolated in 24.1% yield (of SlF-2 mass). The MW of Sl-GF according to HPLC was 51.4 kDa.

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TABLE 1. Quantitative Content of GFr in *S. lappa* Roots, mg/g Air-Dried Raw Material

Compound, group of compounds	Raw material sample*		
	SL-1	SL-2	SL-3
Fructose	23.24 ± 0.21	22.91 ± 0.21	50.15 ± 0.61
1	21.03 ± 0.18	13.14 ± 0.14	47.04 ± 0.51
2	11.43 ± 0.12	13.98 ± 0.15	34.51 ± 0.30
3	2.21 ± 0.03	2.93 ± 0.03	15.68 ± 0.17
4	8.36 ± 0.10	8.81 ± 0.08	50.05 ± 0.61
5	6.76 ± 0.07	11.87 ± 0.14	22.39 ± 0.24
Alcohol-soluble GFr	107.35 ± 1.18	148.24 ± 1.63	383.42 ± 4.21
Water-soluble GFr	430.57 ± 6.03	351.64 ± 3.87	245.21 ± 2.94
Total content of GFr	514.68	476.97	578.27

*Manufacturer: SL-1, Padma AG (Switzerland, batch 52755); SL-2, Padma AG (Switzerland, batch 53756); SL-3, Himalaya Herbal Healthcare (India).

The fructose content in Sl-GF was 96.14 ± 2.42% (resorcinol method); glucose, 0.305 ± 0.006% (glucose-oxidase method). The Glc:Frc ratio was 1:315.2. The IR spectrum of Sl-GF exhibited bands at 819 (pyranose), 874 (β-bond), and 937 cm^{-1} (furanose ring). The overall character of the spectrum agreed with that for other GFr [16]. Total hydrolysis of Sl-GF occurred under very mild conditions (2% oxalic acid). This indicated that the fructose units had the furanose form. The negative optical rotation $[\alpha]_D^{20}$ (–31.4°) was possible with a β-bond between the fructofuranosyl units. Periodate oxidation consumed 0.99 mole of IO_4^-. The Smith degradation products included glycerin, which was possible for (2→1)-type bonds between hexose units in the polymeric chain.

Methylation results were consistent with a linear Sl-GF structure with a terminal glucopyranosyl group because the principal hydrolysis product of Sl-GF permethylate was 3,4,6-tri-*O*-Me-Frc*f*. 1,3,4,6-Tetra-*O*-Me-Frc*f* and 2,3,4,6-tetra-*O*-Me-Glc*p* were detected in trace quantities. The ^{13}C NMR spectrum of Sl-GF showed six strong resonances of fructofuranosyl units at 104.12 (C-2), 82.62 (C-5), 77.46 (C-3), 75.92 (C-4), 63.15 (C-6), and 61.81 ppm (C-1). This also confirmed the linear structure of the isolated polymer [17]. Thus, Sl-GF was a high-molecular-weight linear GFr of the inulin type that consisted of β-(2→1)-bonded fructofuranose units.

The content of alcohol-soluble GFr in *S. lappa* roots was according to spectrophotometric analysis (resorcinol method) 107.35–383.42 mg/g; of water-soluble GFr, 245.21–430.57; total GFr content, 476.97–578.27 (Table 1).

EXPERIMENTAL

Roots of *S. lappa* were supplied by Padma AG [Switzerland, batch 52755 (SL-1 raw material), batch 53756 (SL-2 raw material) and were purchased at a pharmacy chain [Himalaya Herbal Healthcare, India (SL-3 raw material)]. The species was assigned by Dr. T. A. Aseeva (IGEB, SB, RAS). Samples of raw materials were preserved in the Herbarium of the Department of Biologically Active Compounds, IGEB, SB, RAS (No. As/r-74/3-14/0804; As/r-78/5-10/0907; As/r-78/9-11/0908).

Spectroscopic studies were carried out on an SF-2000 spectrophotometer (Lomo) in quartz cuvettes (10 mm). IR spectra were recorded in KBr pellets on an FT-801 IR-Fourier spectrometer (Simeks). Optical rotation was measured on an SM-3 polarimeter (Zagorsk Optico-Mechanical Plant). GC/MS analysis was performed on a 5973 N GC/MS (Agilent Technologies) with a diffusion pump using a PH-Innowax capillary column (30 m/250 μm/0.50 μm). ^{13}C NMR spectra were recorded on a VXR 500S NMR spectrometer (Varian) at operating frequency 125.7 MHz using solutions (1%) of compounds in DMSO-d_6. The standards were fructose, glucose, saccharose (Acros Organics), 1-kestose (Sigma), nystose (Carbosynth Lim.), and 1^F-β-fructofuranosylnystose (Wako Pure Chemical Industries, Ltd.).

Extraction and Isolation of GFr from *S. lappa*. Ground raw material (100 g, sample SL-1) was extracted in a Soxhlet apparatus successively by hexane, $CHCl_3$, and EtOAc. The remaining raw material was extracted by EtOH (80%) on a boiling-water bath (1:20 ratio, 5×). The resulting alcohol extracts were combined and concentrated to an aqueous residue

that was extracted by $CHCl_3$ and EtOAc. The aqueous residue was concentrated to a syrupy consistency and dried in vacuo to afford fraction SlF-1 (18.34 g). The raw material remaining after EtOH extraction was treated with H_2O (1:30 ratio, 5×) at 90°C. The aqueous extract was concentrated to 300–350 mL and precipitated with acetone (1:5). The resulting precipitate was centrifuged (6,000 g), washed with acetone, and dried to afford fraction SlF-2 (34.72 g).

SlF-1. Frc: 67.53 ± 0.74%. **SlF-2.** Frc: 90.33 ± 1.08%. IR spectrum (ν, cm^{-1}): 597, 819, 874, 937, 989, 1038, 1131, 1219, 1331, 1432, 1644, 2932, 1156.

Isolation of Oligomeric GFr. SlF-1 (10 g) was dissolved in H_2O (200 mL) and placed on a column of cation-exchanger KU-2-8 (Reakhim, H^+-form, 3 × 60 cm, H_2O eluent). Next, the effluent was transferred to a column of anion-exchanger ASD-4-5p (Reakhim, Cl^--form, 4 × 50 cm, H_2O eluent). The combined effluent was concentrated in vacuo to 100 mL and transferred to a column of DEAE-cellulose (Reanal, CO_3^{2-}-form, 2 × 40 cm, H_2O eluent). The aqueous effluent was concentrated and separated over Sephadex G-10 (Farmacia, 2 × 80 cm, H_2O eluent). Five compounds were isolated and identified according to methylation as saccharose (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 (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), nystose (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), 1^F-β-fructofuranosylnystose (Frc:Glc 4:1; 3,4,6-tri-*O*-MeFrc*p*—1,3,4,6-tetra-*O*-Me-Frc*p*—2,3,4,6-tetra-*O*-Me-Glc*p* 3:1:1), and 1^F-β-fructofuranosyl-1^F-β-fructofuranosylnystose (Frc:Glc 5:1; 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* 4:1:1).

Total Hydrolysis. The compound (10 mg) was dissolved in TFA (5 mL, 0.5 M) and heated at 120°C for 2 h. The TFA was removed in vacuo in the presence of MeOH. The monosaccharide composition was determined by HPLC [Milikhrom A-02 chromatograph (Ekonova); Separon 5-NH_2 column (Tessek Ltd., 80 × 2 mm, 5 μm); MeCN:H_2O (3:1) mobile phase; ν 0.1 mL/min; 22°C; λ 190 nm].

Methylation was performed by the Ciucanu–Capita method [18] with monitoring by IR spectroscopy. The permethylate was hydrolyzed by HCOOH (90%, 1 h, 100°C) and then TFA (1 M, 4 h, 100°C). The hydrolysate was analyzed by GC/MS.

HPTLC-Densitometric Analysis. HPTLC was carried out on Sorbfil PTSKh-AF-V plates (Imid Ltd.). The test and standard fructose solutions (2 μL each) were deposited as bands (8 mm) using an APA-1 automated applicator (Imid Ltd.). Chromatography was carried out to a height of 6 cm in triplicate at 20°C using PrOH:EtOAc:H_2O (9:7:4) in a vertical chamber that was saturated beforehand with solvent vapor (40 min). Then the plates were dried in a stream of cold air for 20 min, immersed in urea solution (1%) in a mixture of MeOH and H_3PO_4 (10:1), and dried at 110°C for 15 min. Plates were scanned at wavelength 365 nm. A calibration curve was constructed and calculations were performed as before [19].

Isolation of Sl-GF. Fraction SlF-2 (10 g) was dissolved in H_2O (150 mL) and placed on a column of DEAE-cellulose (Reanal, CO_3^{2-}-form, 3 × 50 cm, H_2O eluent). The aqueous fraction was collected, concentrated in vacuo to 100 mL, and treated with acetone (1:10). The resulting precipitate was separated after 2 h by centrifugation (6,000 g) to afford SlF-2′ (7.65 g), a portion of which (5 g) was dissolved in H_2O (100 mL). The resulting solution was placed on a column of Molselect G-25 (Reanal, 3 × 110 cm) and eluted with H_2O. Fractions of 20 mL were collected. Detection used the phenol–H_2SO_4 method [20]. Fractions with the dominant component were combined and precipitated with acetone. The resulting precipitate was centrifuged, dissolved in H_2O (50 mL), and placed on a column of Sephacryl S-300 HR (Sigma, 60 × 1.6 cm). Fractions of 1 mL were collected. The eluent was H_2O. Detection used the phenol–H_2SO_4 method. Fractions in the MW range 65–45 kDa were combined and rechromatographed under analogous conditions over a column of Sephacryl S-300 HR (Sigma, 60 × 2.8 cm) to afford Sl-Gf (2.41 g).

Sl-GF. $[\alpha]_D^{20}$ –31.4° (*c* 1.0, H_2O). Frc: 96.14 ± 2.42%. IR spectrum (ν, cm^{-1}): 599, 819, 874, 937, 990, 1033, 1130, 1218, 1334, 1418, 1454, 1643, 2930, 3384. HPLC (t_R, min): 10.237. MW (Da): 51,422.

HPLC. The molecular weight of Sl-GF was determined using HPLC on a Summit liquid chromatograph (Dionex), TSK gel GMP×1 column (Supelco, 300 × 7.8 mm, 5 μm), H_2O mobile phase, ν 1 mL/min, 20°C, and UVD 170S UV-detector at λ 190 nm. The column was calibrated beforehand using standard dextrans of MWs 2,000; 500; 100; and 40 kDa (Sigma).

Fructose content in Sl-GF was determined by the resorcinol method [21]; glucose, by the glucose-oxidase method using a standard kit (Glucose assay kit, Sigma).

Mild Hydrolysis. The compound (10 mg) was dissolved in oxalic acid (10 mL, 2%) and heated at 100°C for 30 min. Oxalic acid was neutralized by powdered $CaCO_3$. The hydrolysate was analyzed by HPTLC (PrOH:EtOAc:H_2O, 9:7:4) with detection by *p*-hydroxydiphenylphosphate (1%). Fructose and traces of glucose were detected.

Alcohol- and water-soluble GFr were determined quantitatively by a modified resorcinol method [22].

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