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# GLUCOFRUCTANS FROM *Cousinii radians* ROOTS

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*Structures of glucofructans isolated from* Cousinii radians *roots were studied by methylation, periodate oxidation, and IR and $^{13}C$ NMR spectroscopies. It was found that fructofuranose units were bonded to each other by inulin-type β-(2→1)-gylcoside bonds.*

**Key words:** *Cousinii radians*, fractional composition, water-soluble polysaccharides, glucofructans, pectinic substances, hemicelluloses, structure.

*Cousinii radians* is a perennial plant that is widely distributed over all Kyrgyzstan.

We previously determined the carbohydrate composition of this plant [1]. Structures of the polysaccharides have not been reported.

Herein we report the isolation of carbohydrates from this plant and the physicochemical properties of the glucofructans.

Sugars soluble in alcohol (SSA) were first isolated from a single portion of raw material [2] and then water-soluble polysaccharides (WSPS) [3], pectinic substances (PS), and hemicelluloses (HC) [4] (Table 1).

WSPS (18.0%) dominated in the roots compared with the aerial part and were studied further. Hydrolysis and paper chromatography (PC) identified glucose and fructose in them, which indicated they were glucofructans (GF).

Gel chromatography of starting GF over a column of Sephadex G-75 showed that it was heterogeneous. The molecular weights (MW) calculated from a plot of log M*n* vs. elution volume were in the range 10,500-24,000. More homogeneous GF fractions were obtained by fractional precipitation with alcohol from aqueous solutions (Table 2).

The main fractions were F-3 and F-4, which were GF and were called GF-3 and GF-4, respectively. Thus, further studies of the GF used these fractions as examples.

The homogeneity of F-3 and F-4 was studied using gel chromatography. The resulting optical density plots showed that the fractions were homogeneous and gave the MW. Table 3 compares the properties of the GF.

PC of the total-acid hydrolysis products of GF-3 and GF-4 identified fructose and traces of glucose. The fructose content found by the literature method [5] was 98.7 and 97.3%, respectively, of the total mass (Table 3). The specific rotations were negative and indicative of the $\beta$-configuration for the glucoses in the GF [6].

The IR spectra of the polysaccharides had absorption bands at 820, 860, and 940 $cm^{-1}$, which are typical of inulin- and levan-type GF. The absorptions at 820 and 940 $cm^{-1}$ are due to vibrations of pyranose and furanose rings, respectively. The absorption band at 860 $cm^{-1}$ is consistent with $\beta$-glycoside bonds [7].

Periodate oxidation [8] of GF-3 and GF-4 showed that the consumption of sodium periodate remained constant after 120 h at 0.98 and 0.96 mole per mole of anhydrohexose unit. The amount of produced formic acid was 0.043 and 0.042 mole, respectively (Table 3). PC of the Smith degradation products detected glycerine, which indicated that the GF contained $\beta$-(2→1)-glycoside bonds. The polymer chains of GF-3 and GF-4 are not branched at the C-3 and C-4 positions of the anhydrofuranose units.

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TABLE 1. Quantitative Carbohydrate Content in *C. radians*, %

| Plant organ | SSA | WSPS | PS | HC |
|---|---|---|---|---|
| Roots | 9.0 | 18.0 | 4.5 | 6.5 |
| Aerial part | 5.7 | 4.5 | 2.6 | 4.5 |

TABLE 2. Fractional Precipitation of GF by Alcohol

| Parameter | Ethanol solution | | | | | |
|---|---|---|---|---|---|---|
| | 1:1.0 | 1:1.5 | 1:2.0 | 1:2.5 | 1:3.0 | 1:3.5 |
| Ethanol addition, mL | 100 | 150 | 200 | 250 | 300 | 350 |
| Fraction number | F-1 | F-2 | F-3 | F-4 | F-5 | F-6 |
| Yield, % | - | 4.5 | 48.5 | 39.0 | 6.0 | 1.0 |
| MW | - | - | 13800 | 34000 | - | - |

TABLE 3. Comparative Properties of GF-3 and GF-4

| GF | MW | Fructose, % | $[\alpha]_D^{22}$ (*c* 1, water) | Periodate oxidation, mol | |
|---|---|---|---|---|---|
| | | | | $NaIO_4$ | HCOOH |
| GF-3 | 13800 | 98.7 | -39.5 | 0.98 | 0.043 |
| GF-4 | 34000 | 97.3 | -40.0 | 0.96 | 0.042 |

TABLE 4. Chemical Shifts of C Atoms in $^{13}C$ NMR Spectra of GF

| GF | Unit | C-1 | C-2 | C-3 | C-4 | C-5 | C-6 |
|---|---|---|---|---|---|---|---|
| GF-3 | -2-$\beta$-D-Fruf-1- | 62.4 | 104.3 | 78.3 | 75.6 | 82.3 | 62.02 |
| GF-4 | -2-$\beta$-D-Fruf-1- | 61.8 | 104.25 | 78.4 | 75.65 | 82.3 | 62.2 |

The nature of the monosaccharide bonds in the GF was found by Hakomori methylation [9]. Thin-layer chromatography (TLC) showed that the methylation was complete after two methylations. The methylation products of the GF, the permethylates, were obtained in yields of 84.0 and 83.0% with specific rotations $[\alpha]_D^{22}$ of -50.5° and -50.0°, respectively. TLC of the formylosis and hydrolysis products of GF-3 and GF-4 (compared with standards) identified 2,3,4,6-tetra-*O*-Me-D-glucose, 1,3,4,6-tetra-*O*-Me-D-fructose, and 3,4,6-tri-*O*-Me-D-fructose (main product) and traces of 1,3,4-tri-*O*-Me-D-fructose.

Quantitative ratios of methylated sugars found by GC for GF-3 and GF-4 were 1:5:14:0.5 and 1:5:16:0.5, respectively.

Analysis of the methylation products showed that the GF polymeric chain contained $\beta$-(2→1)-bound fructofuranose units. The presence of 3,4,6-tri-*O*-Me-D-fructose in the methylation products as the principal substance confirmed this.

$^{13}C$ NMR spectroscopy also confirmed that there were $\beta$-(2→1)-glycoside bonds between structural units of the macromolecular GF. These gave chemical shifts of 104.25-104.34 ppm for C-2 of the fructofuranose and a signal at 75.6-75.65 ppm for C-4 [10] (Table 4).

Thus, it was found that GF from *C. radians* consist of inulin-type fructofuranose units with $\beta$-(2→1) glycoside bonds. The GF differ from each other in the fructose content and MW.

## EXPERIMENTAL

Solutions were evaporated in a rotary evaporator at 45 ± 5°C. Descending chromatography was performed on FN-7 and -11 paper using the solvent system (v/v) butan-1-ol:pyridine:water (6:4:3). Sugars were developed using anilinium acid phthalate, 10 min, 105-110°C (1); alcoholic urea (5%) (2); and alcoholic bromphenol blue (1%) (3). TLC of methylated sugars was carried out over silica gel KSK 15-5/40 μm and Silufol UV-254 using system 2 (benzene:acetone, 2:1). Sugars and their methylated derivatives were identified using markers. The quantitative ratio of sugars was determined by GC in a Tsvet-101 chromatograph with a flame-ionization detector, stainless-steel column (200 × 0.2 mm) packed with Silicone SE-30 (5%) on Chromaton NAW-DMCS (0.160-0.200 mm), 180-220°C, $N_2$ carrier gas at 40 mL/min. Samples were recorded as trimethylsilyl derivatives.

Specific rotations were measured on a SU-3 saccharimeter in a 1-dm tube of 10 mL volume at 20-23°C. Fructose was determined as before [5].

IR spectra were recorded on a UR-20 instrument in KBr disks; $^{13}C$ NMR spectra, on a Bruker WM-260 instrument at working frequency 62.89 MHz for solutions (3%) of GF in $D_2O$ with methanol internal standard (50.15 ppm).

**Isolation and Purification of GF.** Raw material was collected during fruiting. A weighed portion (200 g) of ground raw material was treated with $CHCl_3$ and ethanol (after isolating oligosaccharides), extracted with water (1:6) for 45 min at 75°C with constant stirring, and filtered. The process was repeated another time. The extracts were combined and concentrated to half the volume. Polysaccharides were precipitated by ethanol (1:3). The resulting precipitates were separated, washed with acetone and ether, and dried. Yield 21.5%.

**MW Determination.** A weighed portion (0.02 g) of polysaccharide was dissolved in water (2 mL) and placed on a column (1.2 × 65 cm) of Sephadex G-75. The column was calibrated by passing dextran samples of MW 40,000 ($V_1$, 16.8 mL), 20,000 ($V_2$, 270 mL), and 10,000 ($V_3$, 31.7 mL). Eluents (2 mL each) were collected and analyzed using phenol:$H_2SO_4$ [11].

**Acid Hydrolysis.** GF (0.1 g) was hydrolyzed in HCl (5 mL, 0.5%) on a water bath for 45 min at 85°C. The hydrolysate was evaporated to dryness in vacuo. PC detected fructose and traces of glucose (system 1, developers 1 and 2).

**Periodate Oxidation and Smith Degradation.** GF-3 and GF-4 (0.2 g) were dissolved in water (50 mL) and treated with sodium periodate (10 mL, 0.25 M). The mixture was kept in the dark at room temperature with constant stirring. Analytical samples were taken each 12 h. The consumption of sodium periodate was determined by titration with sodium thiosulfate solution (0.01 N). The consumption of sodium periodate stopped after 120 h and did not change further.

After the periodate oxidation was finished, the excess of periodate was destroyed by adding ethyleneglycol (3 drops). Then the mixture was reduced with sodium borohydride (0.1 g) and neutralized by KU-2 cation exchanger ($H^+$-form). The solution was concentrated. The dry solid was hydrolyzed by $H_2SO_4$ (2.4 mL, 0.5 N) on a boiling-water bath for 4 h. The mixture was neutralized by barium carbonate and treated with KU-2 ($H^+$-form). The solid was analyzed by PC (system 1, developers 1, 2, and 3). Glycerin was detected.

**Methylation.** A weighed portion (0.02 g) of GF dissolved in DMSO (2 mL) over 90 min on a magnetic stirrer. Sodium hydride (0.01 g) was dissolved separately in DMSO (2 mL) at 40-50°C until a greenish-blue color appeared. The resulting solution was combined with the GF solution, stirred on a magnetic stirrer for 5-6 h under $N_2$, treated with methyliodide (1 mL), left in the dark for 10-12 h, decomposed by adding sodium hyposulfite solution (10%, 3-4 drops), and dialyzed. The solution was extracted with $CHCl_3$ (4 × 5 mL). All extracts were combined and concentrated to a syrup. The completeness of the methylation was monitored by IR spectroscopy (lack of hydroxyl vibrations). The process was repeated twice for exhaustive methylation.

**Formolysis and Hydrolysis of Permethylates.** GF-3 and GF-4 permethylates (0.02 g each) were each hydrolyzed by formic acid (5 mL, 85%) on a boiling-water bath for 1 h, diluted with methanol, and evaporated to dryness. The solid was hydrolyzed by $H_2SO_4$ (2.5 mL, 0.5 N) for 5 h on a boiling-water bath, neutralized by barium carbonate, filtered, treated with KU-2 ($H^+$-form), and concentrated to a syrup. The methylated products were analyzed by TLC using system 2 to identify 2,3,4,6-tetra-*O*-Me-D-glucose, 1,3,4,6-tetra-*O*-Me-D-fructose, 3,4,6-tri-*O*-Me-D-fructose, and traces of 1,3,4-tri-*O*-Me-D-fructose.

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