

A Novel Fructan Possessing DB Value from Roots of *Arctium lappa* L.

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Abstract: A fructan named ALF was isolated from the roots of *Arctium lappa* L.. The structure of ALF was identified by analyses of methylation, GC-MS, and both ^1H and ^{13}C NMR spectroscopy. The results obtained indicated that ALF, comprised of D-fructose and D-glucose in the molar ratio of 14:1, was an inulin-type fructan, which was confirmed by the composition of 14 fructose residues linked by β (2 \rightarrow 1) glycosidic bond and 1 glucose residue linked by α (1 \rightarrow 2) glycosidic bond at the end of linear straight sugar chain.

Keywords: Fructan, Structure, DB (degree of branch) value, *Arctium lappa* L.

INTRODUCTION

Fructans exist as a wide range of oligo- and polysaccharides in many species of bacteria, fungi, and plants [1]. They are classified into different families on the basis of their glycosidic linkages, consisting of (2 \rightarrow 1)-linked β -D-fructofuranosyl units such as inulin, or (2 \rightarrow 6)-linked β -D-fructofuranosyl units such as levans, or highly branched structures comprised of both (2 \rightarrow 1)- and (2 \rightarrow 6)-linked β -D-fructofuranosyl units such as graminans [2, 3].

Arctium lappa L., a fructan-containing member of the *Compositae* family, is a very popular edible vegetable in the orient countries. It has been extensively analyzed for its reserve and cell-wall polysaccharides [4], components having antimicrobial activity as well as for extractive components with antioxidant activity [5]. Kardosová A reported that a water-soluble fructan isolated from *Arctium lappa* L. has antitussive activity [6]. The present work reports on isolation, structural analysis of a fructan from the roots of *A. lappa* L., widely distributed in our geographical conditions.

MATERIALS AND METHODOLOGY

Materials and Instruments

T-series Dextran, DEAE-Sephadex A-50, and DEAE-cellulose were purchased from Amersham Biosciences (Uppsala, Sweden). Trifluoroacetic acid (TFA) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemical reagents were of analytical reagent grade.

NMR spectroscopy: AVANCE-600 superconductive NMR spectrometer (Bruker, Switzerland).

Optical rotation: WZZ-1S automatic polarimeter (Shanghai Physical Optics Instruments Co.).

IR: Perkin-Elmer 591B spectrophotometer with a KBr pellet (native ALF) or Nujol film (permethylated ALF).

GLC: Shimadzu-14B apparatus equipped with a 3% OV-225/AW-DMCS-Chromosorb W column (2.5 m \times 3 mm)

and an FID detector (detector temperature: 250 $^{\circ}\text{C}$); column temperature programming: 110 $^{\circ}\text{C}$ (5 min) \rightarrow 210 $^{\circ}\text{C}$ (20 min).

Element analysis: VarioEL III elemental analyzer.

Plant

The roots of *A. lappa* L. were the product of Gaomi City, Shandong Province, China, in March 2007 and identified by Ji'nan Botanical Garden and Professor Kao-Shan Chen in the School of Life Science, Shandong University (Ji'nan, China).

Isolation of ALF

The in shade air-dried roots (1 kg) were cut into pieces with a fodder chopper and extracted with EtOH (4 L) at room temperature for several days to remove fat and pigment. The residue was extracted successively with H₂O (5 L, 2 h) at 80 $^{\circ}\text{C}$ for three times, filtered through gauze and centrifuged to remove water-insoluble materials. The aqueous extract was concentrated at 45 $^{\circ}\text{C}$ *in vacuo* and treated with 3 vols of 95% EtOH for precipitation at 4 $^{\circ}\text{C}$ overnight. The gel-like precipitate was solubilized in H₂O and dialyzed against distilled H₂O (exclusion limit 0.8 kDa). The nondialyzable portion was treated with papain-Sevag method to remove protein. After the process was repeated two times, the supernatant was lyophilized, and a yellow product was obtained named ALF1.

ALF1 was dissolved in distilled H₂O, centrifuged and loaded onto a DEAE-cellulose column (80 cm \times 3.5 cm). The column was eluted with H₂O. Fractions of 10 mL were collected and monitored for the presence of carbohydrate using the phenol-H₂SO₄ assay. Fractions containing carbohydrate were pooled, dialyzed and lyophilized. The fraction eluted with water from ALF1 designated as ALF2 was further fractionated on a column (100 cm \times 3.5 cm) of Sephadex G-50, eluted with H₂O. The carbohydrate was eluted as one single fraction (ALF) according to the elution profile. The fraction ALF was dialyzed and lyophilized.

Homogeneity and Molecular Weight

High-performance gel permeation chromatography (HPGPC) was carried out with a Waters 515 pump equipped

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Table 1. GC-MS Data for Methylation Analysis of ALF

Methylated Sugars	Molar Ratio	Linkage Types
2, 3, 4, 6-Tetra- <i>O</i> -Me-Glc	1.0	Glc _p (1→
1, 3, 4, 6-Tetra- <i>O</i> -Me-Fru	1.0	Fru _f (2→
3, 4, 6-Tri- <i>O</i> -Me-Fru	13	→1) Fru _f (2→

with a Waters Ultrahydrogel™ 1000 column and a Waters 2410 RI detector. The column was calibrated with standard T-series Dextran (T-500, T-110, T-80, T-70, T-40 and T-9.3) with 0.003 M NaOAc as the mobile phase at a flow rate of 0.5 mL/min. All samples were prepared as 0.4% (w/v) solutions and 20 μL of solution was analyzed in each run. The data were processed with Waters GPC Millennium³² software.

Monosaccharide Analysis and Linkage Analysis [7]

ALF (3 mg) was hydrolyzed with 2 M TFA at 80 °C for 1 h, followed by evaporation to dryness. The residue was redissolved in H₂O (0.2 mL), with 5 μL of the solution used for TLC analysis. The other portion was successively reduced with NaBH₄, acetylated with Ac₂O at 100 °C for 1 h, and the resulting alditol acetate was examined by GLC.

ALF (5 mg) was methylated four times using the modified Ciucanu method [8]. The permethylated ALF was depolymerized with 90% formic acid (100 °C, 1 h), followed by hydrolysis with 2 M TFA (100 °C, 2 h). The hydrolysate was converted into partially methylated alditol acetate and analyzed by GC-MS with a Shimadzu QP Class-5000 instrument.

¹H NMR and ¹³C NMR Spectroscopy

¹H and ¹³C NMR spectra were measured using a Bruker AM-400 NMR instrument equipped with a dual probe in the FT mode at 20 °C. ALF (30 mg) was dissolved in D₂O at a concentration of 30 mg/0.5 mL. Chemical shifts are referred

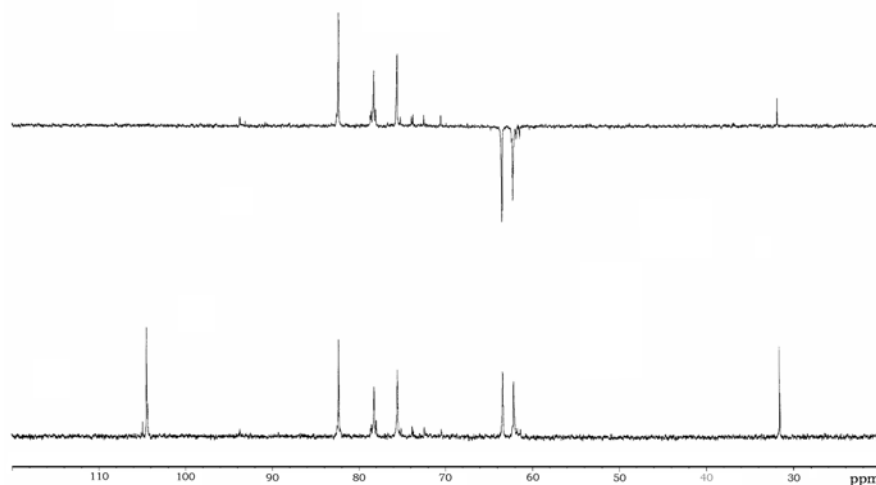
to the residual signal of HOD at δ 4.70 ppm for ¹H NMR spectrum and the internal standard, acetone for ¹³C NMR spectra.

RESULTS AND DISCUSSION

ALF was obtained from the root of *A. lappa*, using water extraction, DEAE-cellulose column, and Sephadex G-50. On HPGPC, ALF showed a symmetrical peak, indicating a homogenous fraction. The average molecular weight was estimated to be 3.1×10^3 g/mol. After complete hydrolysis with 2 M TFA, TLC analysis showed that ALF contains no uronic acid. GLC analysis indicated that it was composed of fructose and glucose. The absorption in the IR (not shown) indicated that ALF has characteristic peaks of polysaccharide. The content of C (40.53 %) and H (6.599 %) was estimated by elemental analysis. The molar ratio of C: H: O was 1:2:1. The $[\alpha]_D$ value of ALF was -33 (*c* 0.80, H₂O).

After methylating four times using the modified Ciucanu method, the O-H absorption at 3600-3200 cm⁻¹ in IR disappeared, indicating the completeness of methylation. The permethylated ALF was depolymerized and converted into partially methylated alditol acetate. GC-MS analysis showed three types of linkages, corresponding to T-Glc_p (terminal-Glc_p), →1) Fru_f (2→ and Fru_f (2→, respectively, approximately in the molar ratio of 1:13:1 (Table 1).

The ¹³C NMR spectrum contained two anomeric signals at δ 104.48 ppm and δ 93.58 ppm. The signal at δ 104.48 arose from the anomeric atoms of →1) Fru_f (2→ and the signal at δ 93.58 was assigned to T-Glc_p. The corresponding

**Fig. (1).** ¹³C NMR spectrum of ALF.

