

STRUCTURE OF A GLUCOGALACTAN FROM LEAVES OF *Ficus carica*

M. O. Kuronboeva,^{1*} K. S. Zhauynbaeva,^{1*}
R. K. Rakhmanberdyeva,¹ and M. R. Mirzaeva²

A glucogalactan with molecular mass 36 kDa and glucose:galactose ratio of 1.0:1.54 was isolated from leaves of Ficus carica L. The structure of the glucogalactan was studied by chromic oxidation, methylation, and ¹³C NMR spectroscopy. The main chain of the GG-Fc glucogalactan macromolecule included mainly β-1,3-bonded galactopyranose residues, several of which had C-4 side branches. The glucose residues were 1→6, 1→4, and 1→3 bonded via glycoside bonds. Galactose and glucose residues were found on the unreduced terminuses.

Keywords: *Ficus carica* L., glucogalactan, methylation, chromic oxidation, IR, GC-MS, and ¹³C NMR spectroscopy.

The plant *Ficus carica* L. (Moraceae) (fig) is broadly distributed in the Republic of Uzbekistan. Various fig varieties are widely cultivated in all regions of the Republic, while the wild species grow in Surxondaryo Region [1].

According to the literature, fig leaves are rich in furocoumarins and have been used to create the preparation psoboran (mixture of psoralen and bergapten furocoumarins) to treat vitiligo [2]. The decoction of leaves and roots is used in folk medicine for stomach disorders. The carbohydrate composition and biological activity of polysaccharides from this plant species are poorly studied. Previously, the carbohydrate composition of fig leaves was reported by us [3]. Herein, results from chemical studies of the water-soluble polysaccharides (WSPS) and their biological activity are reported.

Paper chromatography (PC) and gas chromatography (GC) of the hydrolysate of the WSPS detected rhamnose, xylose, arabinose, glucose, and galactose in a 1.0:2.3:2.4:3.4:5.3 ratio. The content of uronic acids (34%) was determined by the carbazole method. Therefore, the starting WSPS were a mixture of neutral and acidic polysaccharides. They were separated by passing the starting WSPS over a column of DEAE cellulose (OH-form). Elution by H₂O isolated neutral polysaccharides (NPS) in 35% yield. Their monosaccharide composition included galactose, glucose, and traces of arabinose. Next, the column was eluted with NaOH solution (0.1 N) to isolate acidic polysaccharides (APS) in 5.3% yield. They consisted of arabinose, glucose, galactose, and uronic acid. A homogeneous fraction of NPS was produced by fractionation with EtOH of the aqueous solution to afford four fractions (Table 1). Table 1 shows that the monosaccharide compositions of all fractions included glucose, galactose, and traces of arabinose. Only fraction III, which gave the greatest yield, was homogeneous using gel chromatography over a column of Sephadex G-50. Therefore, it was studied further and was designated glucogalactan GG-Fc.

The IR spectrum of glucogalactan GG-Fc showed characteristic absorption bands at 3259, 2300, 1567, 1410, 1195, 1086, 1045, 880, and 599 cm⁻¹. The absorption band at 880 cm⁻¹ was indicative of a β-glycoside bond between monosaccharide residues. A method based on oxidation of the fully acetylated polysaccharide by chromic anhydride in glacial AcOH was used by us to confirm this hypothesis [4]. An analysis of the reaction products showed that all sugar residues were oxidized (lack of monosaccharides in the hydrolysate), indicative of a β-glycoside bond between the monosaccharides.

The types of glycoside bonds between the monosaccharide residues were established via methylation of GG-Fc by the literature method [5]. The hydroxyl groups were converted into methyl ethers by methyl iodide and base in DMSO.

1) S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, 77 Mirzo Ulugbek St., Tashkent, 100170, Uzbekistan, e-mail: kzhaunbaeva@inbox.ru; mohinurkuranbaeva67@gmail.com;
2) Osh State University, Department of Chemistry and Chemical Technology. Translated from *Khimiya Prirodnikh Soedinenii*, No. 2, March–April, 2025, pp. 185–187. Original article submitted July 2, 2024.

TABLE 1. Characteristics of NPS Fractions

Fraction	Solution:EtOH ratio	Yield, % of NPS	MM, kDa	Monosaccharide composition, GC	
				Glc	Gal
I	1:0.5	16.4	42	1	1.3
II	1:1	19.1	40	1.0	1.36
III	1:1.5	36.4	36	1.0	1.54
IV	1:2	5.7	20	1	2.0

TABLE 2. Analysis of Hydrolysate of Permethylate of GG-Fc Glucogalactan by GC-MS

Acetate of partially methylated polyols	Structure fragment	Content, %
2,3,4,6-tetra-OMe-Galp	β -Galp1) $\rightarrow$	25.9
2,4,6-tri-OMe-Galp	$\rightarrow$ 3)- β -Galp-(1 $\rightarrow$	25.2
2,6-di-OMe-Galp	$\rightarrow$ 3,4)- β -Galp-(1 $\rightarrow$	10.1
2,3,4,6-tetra-OMe-Glcp	β -Glcp-(1 $\rightarrow$	18.3
2,6-di-OMe-Glcp	$\rightarrow$ 3,4)- β -Glcp1 $\rightarrow$	10.3
2,3,6-tri-OMe-Glcp	$\rightarrow$ 4)- β -Glcp-(1 $\rightarrow$	2.5
2,3,4-tri-OMe-Glcp	$\rightarrow$ 6)- β -Glcp-(1 $\rightarrow$	7.3

The completeness of the methylation was monitored by IR spectroscopy and the lack of an absorption band for the hydroxyls. The permethylate was formolyzed and hydrolyzed. The products were reduced by NaBH₄ and acetylated. Sugars were identified by GC-MS. Table 2 presents the results.

The presence of 2,4,6-tri-OMe-Galp as the main product indicated that the main polymer chain in the GG-Fc macromolecule consisted of β -1,3-bonded galactopyranose residues. Isolation of 2,6-di-OMe-Galp was consistent with the main chain being branched at C-4. Detection of 2,3,4-tri-OMe-Glcp, 2,3,6-tri-OMe-Glcp, and 2,6-di-OMe-Glcp provided evidence that the glucose residues could have 1 $\rightarrow$ 6, 1 $\rightarrow$ 4, and 1 $\rightarrow$ 3 glycoside bonds. The presence of 2,3,4,6-tetra-OMe-Galp and 2,3,4,6-tetra-OMe-Glcp indicated that galactose and glucose residues occupied the unreduced terminuses. The structure of glucogalactan GG-Fc was studied by ¹³C NMR spectroscopy. Resonances at 84.2 and 83.8 ppm were attributed to 1,3-bonded galactose residues; at 69.1 ppm, to 1,6-bonded glucose residues. Resonances at 81.9 and 79.3 ppm confirmed that galactose and glucose C-4 were branching sites.

The biological activity of *F. carica* WSPS in a formalin arthritis model found that the WSPS exhibited pronounced anti-inflammatory activity at a dose of 150 mg/kg (58%), which was inferior to the effect of ketoprofen at a dose of 5 mg/kg (70%) and superior to diclofenac at the same dose (50%) [3].

Thus, the branched glucogalactan GG-Fc was isolated for the first time from leaves of *F. carica*. The main chain of the GG-Fc macromolecule included primarily β -1,3-bonded galactopyranose residues, some of which had C-4 side branches. The glucose residues in the molecules were connected by 1 $\rightarrow$ 6, 1 $\rightarrow$ 4, and 1 $\rightarrow$ 3 glycoside bonds. The unreduced terminuses held galactose and glucose residues. The WSPS from *F. carica* exhibited a pronounced anti-inflammatory effect.

EXPERIMENTAL

IR spectra were recorded in KBr pellets on an FTIR spectrometer (PerkinElmer). GC of the samples used a GC 2010 Plus chromatograph (Shimadzu) with an APC flame-ionization detector (FID) (Det3ch). Chromatography used an Rxi-624Sil MS glass capillary column (30.0 m $\times$ 0.25 mm, 1.40 μ m). The carrier gas was N₂. The total carrier-gas flow rate was 30 mL/min; flow through the column, 0.71 mL/min; vaporizer temperature, 260°C; column, 230°C; detector, 280°C. A solution containing aldonitrile acetates (1 μ L, 10 mg/mL, 0.01%) was injected into the chromatograph vaporizer. The retention times of the constituents and the mixture compositions were measured by computer on the recorded chromatograms. NMR spectra

were recorded on an Avance AV600 spectrometer (Bruker, Germany) at operating frequency 600 MHz. Samples were dissolved in D₂O according to the company standard procedures. Chemical shifts were calculated using TSP as an internal standard (δ_{H} 0.0, δ_{C} 1.6).

Paper chromatography used Filtrak-FN 13,18 paper in descending mode and the solvent system *n*-BuOH–Py–H₂O (6:4:3). Spots were detected using aniline hydrogen phthalate.

Leaves of *F. carica* were collected in September 2022 in Tashkent Region.

Isolation of WSPS. Ground leaves of *F. carica* (100 g) were worked up twice in refluxing CHCl₃ (1:5 and 1:3). The raw material was separated by filtration, dried, and extracted twice with refluxing EtOH for 1 h (1:5 and 1:3). The remaining raw material was extracted (3×) with H₂O for 2 h at room temperature (1:10, 1:5, 1:5). The extracts were combined, centrifuged, and condensed to 200 mL. The resulting precipitate was separated by centrifugation, rinsed with EtOH, dehydrated by Me₂CO, and dried under vacuum. The yield of water-insoluble polysaccharide (WISPS) was 0.44 g. The supernatant solution from isolation of WISPS was precipitated by adding three times the volume of EtOH. The resulting precipitate was worked up analogously as above to afford WSPS (3.55 g).

Separation of WSPS Over a Column of DEAE-cellulose (OH[−]-form). DEAE-cellulose (100 g) was successively worked up with HCl solution (0.5 N), H₂O until neutral, NaOH solution (0.5 N), and H₂O until neutral. It was placed into a column (4.5 × 40 cm). WSPS (2.8 g) were dissolved in H₂O (100 mL), placed onto the column, and eluted with H₂O (3 L). The eluate was condensed to 100 mL and precipitated with EtOH (1:5). The precipitate was separated by centrifugation (5000 rpm, 10 min), rinsed, and dried by EtOH. Yield of NPS, 2.25 g. Next, the column was eluted by NaOH solution (0.1 N). The basic eluate containing acidic polysaccharides was neutralized with AcOH, dialyzed against distilled H₂O, condensed, and precipitated with EtOH (1:5). Yield of acidic polysaccharides (APS), 0.34 g.

Fractionation of NPS. NPS (2.25 g) were dissolved in H₂O (100 mL) and treated dropwise with vigorous stirring with EtOH (50 mL). The resulting precipitate was separated by centrifugation (5000 rpm, 10 min), rinsed with EtOH, and dried. Yield 0.37 g (fraction I). The supernatant solution was treated with more EtOH (50 mL). The precipitate was separated as above. Yield 0.43 g (fraction II). Fraction III was isolated by adding EtOH (50 mL). The yield was 0.82 g. Yield of fraction IV, 0.13 g.

Hydrolysis. WSPS and fractions I–IV (100 mg each) were hydrolyzed by H₂SO₄ solution (3 mL, 1 N) for 7–8 h at 100°C. The hydrolysates were neutralized with BaCO₃, deionized by KU-2 cation exchanger (H⁺-form), evaporated, and analyzed by PC and GC as the aldonitrile acetates. The standards were the monosaccharides Rha, Xyl, Ara, Glc, Man, Gal, and GalUA.

Uronic acid contents of WSPS were determined by the carbazole method [6].

Gel chromatography was performed over a column of Sephadex G-50 (52 × 1.8 cm). The column was calibrated with dextrans of molecular masses 15,000–20,000 (Ve₁, 45 mL; Ve₂, 39 mL) and 40,000 (Ve₃, 21 mL). The eluent was distilled H₂O. Aliquots (3 mL) were collected. Fractions I–IV (10 mg each) were dissolved in distilled H₂O (2 mL) and placed onto the column. The yields of polysaccharides were monitored by the phenol–H₂SO₄ method [7]. Molecular masses were determined from a calibration curve for the dependence of the elution volume Ve on the logarithm. Ve₄, 18 mL (starting WSPS); Ve₅, 18 mL (fraction I); Ve₆, 21 mL (fraction II); Ve₇, 27 mL (fraction III); Ve₈, 39 mL (fraction IV).

Acetylation. GG-Fc (0.1 g) was dissolved in formamide (10 mL) and treated with Py (10 mL) and Ac₂O (20 mL). The reaction mixture was held at room temperature for 48 h and precipitated with ice water (200 mL). The precipitate was separated and dried. The yield of GG-Fc peracetate was 0.084 g. The IR spectrum lacked an absorption band at 3200–3600 cm^{−1} (OH groups) and had clearly pronounced absorption bands at 1740 and 1250 cm^{−1}.

Chromic Oxidation. CrO₃ (0.5 g) was dissolved in glacial AcOH (15 mL) and treated with GG-Fc peracetate (0.05 g). The mixture was heated for 4 h at 50°C, diluted with H₂O, and extracted with CHCl₃. The extract was dried over anhydrous Na₂SO₄ and evaporated to dryness. The solid was dissolved in H₂SO₄ solution (1 mL, 1 N) and hydrolyzed for 16 h on a boiling-water bath. The hydrolysate was worked up as usual. PC (system I, developer I) did not detect monosaccharides.

Methylation. GG-Fc (0.1 g) was dissolved in DMSO (4 mL) and treated with MeI (2 mL), and NaOH powder (30 mg). The mixture was stirred on a magnetic stirrer for 2–3 h at room temperature, diluted with H₂O (4 mL), treated with CHCl₃ (4 mL), and shaken. The CHCl₃ layer was separated, dialyzed, dried over Na₂SO₄, filtered, and evaporated to dryness. The completeness of the methylation was monitored from the lack of an absorption band for hydroxyls at 3400–3600 cm^{−1} in the IR spectrum. The methylated product was dissolved in formic acid (2 mL), heated for 1 h at 100°C, and evaporated to dryness. The solid was treated with trifluoroacetic acid (2 mL, 2 M) and heated for another 4 h at 100°C. The resulting solution was evaporated to dryness. The solid was dissolved in NH₄OH solution (1 mL, 1 M), reduced with NaBH₄, worked up with

KU-2 cation exchanger (H^+ -form), filtered, and evaporated to dryness. The dry solid was treated with anhydrous Py (1 mL) and Ac_2O (2 mL) and heated at $90^\circ C$ for 1 h. The reaction mixture was treated with H_2O (4 mL) and worked up with $CHCl_3$. The $CHCl_3$ layer was separated, washed with H_2O , dried over Na_2SO_4 , filtered, and evaporated to dryness. The resulting mixture was analyzed by GC-MS.

ACKNOWLEDGMENT

The work was supported by State Base Financing from the Republic of Uzbekistan.

REFERENCES

1. Kh. Kh. Khalmatov, A. Kh. Usmankhodzhaev, M. I. Makhsumov, and U. A. Akhmedov, *Atlas of Medicinal Plants* [in Russian], Tashkent, 1998, p. 72.
2. B. S. Okhundedaev, Kh. M. Bobokulov, R. F. Mukhamatkhanova, S. Z. Nishanbaev, and I. Dzh. Sham'yanov, *Farm. Zh.*, **29**, 12 (2020).
3. M. A. Kuronboeva, K. S. Zhaunbaeva, A. A. Azamatov, and F. M. Tursunkhodzhaeva, *Polysaccharides of Ficus carica, Actual Problems of the Chemistry of Natural Compounds*, Abstracts, Mar. 15–16, Tashkent, 2023, p. 169.
4. F. A. Kodiralieva, A. S. Shashkov, and R. K. Rakhmanberdyeva, *Chem. Nat. Compd.*, **51**, 405 (2015).
5. R. K. Rakhmanberdyeva, D. Z. Azizov, and A. S. Dmitrenok, *Chem. Nat. Compd.*, **59**, 825 (2023).
6. V. V. Arasimovich (ed.), *Biochemical Methods of Fruit Analysis* [in Russian], Shtiintsa, Kishinev, 1984, p. 12.
7. M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.*, **28** (3), 350 (1956).