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CARBOHYDRATES AND LIPIDS OF *Gleditsia triacanthos* SEEDS

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The carbohydrate and lipid compositions of seeds from Gleditsia triacanthos *L. cultivated in Uzbekistan are investigated. Water-soluble polysaccharide (galactomannan), pectinic substances, and hemicellulose are isolated. Their physicochemical properties and monosaccharide composition are found. The content and fatty-acid composition of neutral, glyco-, and phospholipids are determined.*

Key words: *Gleditsia triacanthos*, Cesalpinaceae, carbohydrates, lipids.

Gleditsia triacanthos L. (Cesalpinaceae) grows in the southern part of European Russia, Crimea, the Caucuses, and Central Asia [1]. It is cultivated in gardens and parks in countries with a temperate climate. The plant contains alkaloids flavonic pigments, anthroglycosides, and tanning agents [2]. It is used in folk medicine for spastic colitis, chronic cholecystitis, stomach ulcers, and bronchial asthma [3].

We continued the investigation of carbohydrates and lipids of seeds of *Gleditsia* cultivated in Uzbekistan [4, 5]. Lipids and carbohydrates were successively isolated from one batch of starting material. The seeds were ground. Lipids were extracted by soaking in a $CHCl_3$—CH_3OH mixture at room temperature. The pulp was dried and used for subsequent extraction of various types of carbohydrates: water-soluble polysaccharides (WSPS), pectinic substances (PS), and hemicellulose (HC). Carbohydrates were hydrolyzed completely by acid. The qualitative and quantitative compositions of the isolated carbohydrates (Table 1) indicate that WSPS are dominate whereas PS and HC are present in equal amounts in *G. triacanthos* seeds.

WSPS are isolated as a white powder with a cream-colored tint. They are freely soluble in water and form thick solutions of relative viscosity 101.5 (*c* 0.5%, H_2O) and 1.7% N content. According to paper chromatography (PC), WSPS consist of galactose and mannose in a 1:4.6 ratio. Therefore, WSPS of *G. triacanthos* seeds are galactomannans. Pure galactomannan without protein impurity was isolated from the seed coating in 25% yield (of the air-dried material). The constancy of the quantitative ratio of galactose and mannose at all isolation stages was taken as the criterion of galactomannan homogeneity. The IR spectra of galactomannan exhibit absorption bands at 820 and 870 cm^{-1}, which correspond to C–H deformations and indicate that the mannose fragments in the galactomannans have the pyranose form and are 1→4 bonded by glycoside bonds [5, 6]. An absorption band at 720 cm^{-1} corresponds to α-D-galactose pyranose ring vibrations [6].

Pectinic substances are isolated from *G. triacanthos* seeds as a light brown powder that is partially soluble in water and completely soluble in base. Total acid hydrolysis of the PS produces mannose, galactose, rhamnose, and galacturonic acid according to PC. The ratio of monosaccharides is determined by GLC (Table 1). The main neutral monosaccharide component of PS is mannose.

The uronic anhydride content according to the carbazole method [7] is 40.5%. The presence of free (A_f) and esterfied (A_e) groups is 3.2 and 5.8%, respectively, as determined by titration [8]. The degree of esterification (λ) in PS is 61.7%. Therefore, the studied PS are highly esterified PS. Hemicellulose is a dark brown powder. Aqueous solutions of HC do not react with starch. The hydrolysate contains rhamnose, mannose, galactose, and galacturonic acid according to PC. According to GLC (Table 1), the main components are mannose and galactose. Therefore, the HC is based on galactomannan.

Lipids were extracted by the Folch method from ground seeds with 8.8% moisture [9]. The lipid content was 3.3% (dry weight). The acid number was 7.3 mg KOH. The total lipids contain carotinoids (6.4 mg%) and unsaponified substances (8.1%).

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TABLE 1. Carbohydrate Content and Monosaccharide Composition of *G. triacanthos* L. Seeds

PS type	PS yield, %	Monosaccharide composition						
		Gal	Glc	Man	Ara	Xyl	Rham	UAc
WSPS	15.8	1.0	-	4.6	-	-	-	-
PS	7.0	4.0	-	21.7	-	-	1.0	+
HC	7.0	2.3	-	6.9	-	-	1.0	+

TABLE 2. Fatty-Acid Composition of Lipids of *G. triacanthos* L. Seeds, % GLC

Acid	NL	GL	PL
12:0	0.1	0.1	0.1
14:0	0.1	0.1	Tr.
16:0	34.4	29.9	29.7
18:0	16.5	15.9	20.4
18:1	38.4	11.2	17.3
18:2	9.0	39.5	31.7
18:3	1.5	3.3	0.8
Σ_{sat}	51.1	46.0	50.2
Σ_{unsat}	48.9	54.0	49.8

Chromatography of total lipids over a silica-gel column isolated neutral lipids (NL, 90.5%), glycolipids (GL, 6.4%), and phospholipids (PL, 3.1%).

The component composition of the lipids was determined using analytical TLC with the conditions used to separate NL, GL, and PL [9]. Lipids were identified by comparing their chromatographic mobilities with those of authentic substances, qualitative reactions, and literature data.

Analysis of NL over a thin-layer of silica gel using solvent systems 1-3 showed the presence of hydrocarbons, triacylglycerides, carotinoids, fatty-acid esters, free fatty acids, sterols, and diacylglycerides.

The GL were (system 4) mono- and digalactosyldiacylglycerides, sterolglycosides, and sterolglycoside esters.

The PL consist (system 5) of phosphatidylcholines, phosphatidylethanolamines, phosphatidylinosites, and traces of phosphatidic acids.

Fatty acids obtained by hydrolysis of NL, GL, and PL were converted to methyl esters. GLC determined their composition (Table 2). It can be seen that the fatty-acid composition of the lipids is close to that of the related species *G. maculata* H.G. et K. [10]. The set of fatty acids in all lipid classes is identical. It differs only in the content of the separate components. The principal acids of NL are palmitic and oleic. The highest content of linoleic acid was observed in GL and PL; the lowest, in NL. Linolenic acid was present in all lipid groups in small amounts (0.8-3.3%).

EXPERIMENTAL

IR spectra were recorded on a Perkin—Elmer 2000 IR-Fourier spectrometer in pressed KBr pellets.

GLC was performed in Chrom-4 and Chrom-5 instruments with flame-ionization detectors using:

a) steel column (0.3×200 cm) packed with XE-60 (5%) on chromaton N-AW (0.200-0.250 mm), carrier gas He (60 mL/min), 200°C (for carbohydrates);

b) steel column (2.5×4 mm), polyethyleneglycolsuccinate (17%) stationary phase on celite-545 (800-100 mesh), carrier gas N_2, evaporator temperature 250°C, thermostat 198°C (for fatty acid methyl esters).

The viscosity of polysaccharides was measured in an Ostwald viscometer. PC of carbohydrates was performed on

Filtrak FN 3 and 12 paper in descending mode using 1-butanol—pyridine—water (6:4:3) with anilinium acid phthalate developer.

Analytical TLC of lipids was carried out over silica gel (5/40) with $CaSO_4$ (10%). The solvent systems were: C_6H_{14}—$(C_2H_5)_2O$ (9:1, 1; 7:3, 2; 3:2, 3), $CHCl_3$—$(CH_3)_2CO$—CH_3OH—CH_3CO_2H—H_2O (65:20:10:10:3, 4), and $CHCl_3$—CH_3OH—NH_4OH (25%) (13:7:1, 5). The developers were iodine vapor, H_2SO_4 (50% for NL), α-naphthol and H_2SO_4 (50%) (for GL), Vas′kovskii reagent, Dragendorff's solution, and ninhydrin (for PL).

Lipids were extracted from seeds using the Folch method [9]. Literature methods were used to purify lipids of ballast material using an aqueous solution (0.04%), identify lipid classes, isolate unsaponified substances, obtain fatty acids, and methylate them using diazomethane [9, 11].

The total lipids were separated by column chromatography over silica gel (160-250 mesh). NL were eluted by $CHCl_3$; GL by $(CH_3)_2CO$; PL by CH_3OH.

WSPS, PS, and HC were isolated from raw material remaining after isolation of lipids according to published methods [4, 5]. Polysaccharides were hydrolyzed by H_2SO_4 (2 N) at 100°C; WSPS, 6 h; PS, 48 h; HC, 72 h.

The content of uronic anhydride and degree of esterification in PS were determined as before [7, 8].

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