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Allium CARBOHYDRATES. XV. POLYSACCHARIDES FROM *Allium motor*

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The content of carbohydrates in Allium motor *was studied as a function of vegetation period. The qualitative and quantitative compositions and physical chemical properties of sugars soluble in alcohol, water-soluble polysaccharides, pectinic substances, and hemicellulose were characterized. Galacturonan was produced by partial hydrolysis of the pectinic substances.*

Key words: *Allium motor*, carbohydrates, water-soluble polysaccharides, glucofructans, pectinic substances, hemicellulose.

In continuation of research on carbohydrates from plants of the Alliaceae family [1], we studied the carbohydrate composition of seven samples of *Allium motor* L. growing in Parkent region of Tashkent district as a function of vegetative period.

A single portion of the plant that had been treated with $CHCl_3$ to remove extractable substances (ES) was treated successively with alcohol (80%) to isolate sugars soluble in alcohol (SSA); distilled water, water-soluble polysaccharides (WSPS); a mixture of $H_2C_2O_4$—$(NH_4)_2C_2O_4$ solutions (0.5%), pectinic substances (PS); and NaOH solution (10%), hemicellulose (HC). Table 1 gives the results.

Table 1 shows that the content of WSPS, PS, and HC increased as a function of *A. motor* vegetative period during senescence whereas that of SSA decreased. The ratio of monosaccharides in them changed accordingly.

Paper chromatography (PC) detected fructose, glucose, saccharose, and oligosaccharides containing fructose in all samples of SSA from bulbs and the aerial part.

The $SSA_{(1)}$ were characterized by column chromatography over Sephadex G-25 (Table 1). Six fractions were obtained. Table 2 gives the results from acid hydrolysis and the physical chemical properties.

Table 2 shows that the $SSA_{(1)}$ from *A. motor* contained a heptasaccharide, stachyose, a trisaccharide, glucose, and fructose.

Removal of proteins by the Sevag method produced WSPS as light cream-colored powders that did not act as reductants and did not give a color reaction with iodine. They were very soluble in cold water to form transparent and nonviscous solutions. PC of the hydrolysates of all WSPS samples detected mainly fructose and traces of glucose. Therefore, the WSPS were glucofructans (GF).

Gel chromatography over Sephadex G-75 showed that two fractions $GF_{6,7}$ (Table 1) were homogeneous whereas the others contained several components with molecular weights that varied from 500 to 40,000 [2, 3]. The IR spectra of the two homogeneous $GF_{6,7}$ (Table 1) contained absorption bands at 820, 860, and 940 cm^{-1}, typical of 2→1 bonds in the fructofuranose units and similar to the absorption bands of inulin [4].

The microelements in homogeneous $GF_{6,7}$ were determined by atomic absorption spectrophotometry on a Perkin—Elmer 403 instrument using the analytical line at 283.3 nm [5]. The following values were found: Ca 0.0059 mg/g, Mn 0.0062, Cu 0.001, Ti 0.0017, Zn 0.0016, Au 0.0003. These microelements are essential for the organism; participate in carbohydrate, protein, and phosphorus exchange; and are found in enzymes that catalyzed redox reactions [6].

The PS were fibrous cream-colored powders that were poorly soluble in water. Samples of PS were subjected to total acid hydrolysis. The hydrolysates were analyzed by PC, which detected glucose, galactose, rhamnose, arabinose, xylose, and galacturonic acid with spots of various intensities.

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TABLE 1. Carbohydrate Components of *Allium motor*, % (abs. dry wt.)

Sample	Plant organ	Development phase and collection date	ES	SSA	WSPS (GF)	PS	HC
1	Aerial part	Start of vegetation, March	2.21	54.3	3.3	7.9	1.30
2	Bulbs	Start of vegetation, March	0.80	46.2	4.4	8.2	1.47
3	Aerial part	Vegetative period, April	2.07	51.4	3.8	8.7	1.64
4	Bulbs	Vegetative period, April	0.69	42.8	4.7	9.1	1.59
5	Bulbs	Fruiting, end of May	0.61	38.7	6.2	10.7	1.81
6	Bulbs	Senescence, August	0.14	19.8	9.7	12.4	2.35
7	Bulbs	Senescence, November	0.07	11.4	16.3	15.3	3.77

TABLE 2. Properties of *A. motor* $SSA_{(1)}$

$SSA_{(1)}$ fraction	SSA-1	SSA-2	SSA-3	SSA-4	SSA-5	SSA-6
Yield, %	10.6	12.7	14.8	17.6	21.4	22.9
Molecular weight	1100	650	504	320	180	180
Monosaccharide composition	Glc, Fru	Glc, Fru, Gal	Glc, Fru	Glc, Fru	Fru	Glc
$[\alpha]_D^{22}$ (*c* 2.0; H_2O)	-39°	+138°	+28°	+66°	-92°	+52.9°

TABLE 3. Physical Chemical Properties of PS

PS sample	Molecular weight	C 0.1:1% NaOH	Uronic anhydride content, %	K_a, %	K_e, %	λ, %	OCH_3, %
1	34800	0.21	46.7	4.74	1.36	19.1	1.31
2	36700	0.22	48.1	4.72	1.38	19.9	1.36
3	38000	0.23	52.8	5.08	1.41	24.1	1.49
4	47000	0.26	54.1	4.63	1.47	23.7	1.58
5	55600	0.34	55.6	5.85	1.58	27.2	1.64
6	68000	0.36	57.2	3.26	1.64	38.6	1.73
7	80000	0.41	58.3	2.20	1.71	37.1	1.76

IR spectra of PS (samples 6 and 7, Table 1) contained characteristic absorption bands [7] at 840 cm^{-1} (α-configuration), 1150 cm^{-1} (maximum degree of esterification), and 815, 870, and 910 cm^{-1} (triplets for pyranose rings). These were consistent with α-1→4 bonds between galacturonic acid and the monosaccharides.

The molecular weights of the PS were determined by viscosimetry [8] (Table 3).

Partial hydrolysis of PS (sample 7, Table 1) produced galacturonan. PC of the hydrolysate detected only galacturonic acid. The high positive specific rotation of the galacturonan $[\alpha]_D^{20}$ +230° (*c* 0.3, 0.1 N NaOH) also suggested that there were glycosidic bonds with the α-configuration between galacturonic acid units in the pyranose form.

HC were light brown powders that were insoluble in water. PC of the hydrolysates detected galactose, glucose, arabinose, xylose, and traces of rhamnose.

Table 2 shows that the PS were slightly methoxylated. During plant growth their molecular weight was low. However, during senescence they and the degree of esterification increased.

EXPERIMENTAL

Solutions were evaporated in a rotary evaporator at 40 ± 5°C. IR spectra were recorded on a UR-20 instrument in KBr disks. PC was performed on Filtrak-FN-7,17 paper using the solvent systems water-saturated phenol (1) and butan-1-ol:pyridine:water (6:4:3, 2).

Spots were developed using alcoholic urea (5%) and anilinium biphthalate.

PS were determined by titration [9]. Specific rotations were measured in a Zeiss polarimeter with a 1-dm tube of volume 10 cc at 20 ± 2°C.

Isolation of Carbohydrate Components. Dry ground raw material (100 g) was treated with $CHCl_3$ and extracted with 96° and 80° alcohol with boiling. The combined extracts (SSA) were evaporated to a thick syrup, purified with carbon, and chromatographed on paper (system 1, developer 1).

The yield of SSA was 54.3 g (Table 1). SSA were chromatographed over a column (35 × 2.5 cm) of Sephadex G-25. Effluents were collected (3 mL/15 min) and analyzed using phenol—sulfuric acid [2]. Six fractions were obtained in effluents with Ve = 198, 214, 236, 248, and 260 mL. The products of their acid hydrolysis contained glucose and fructose according to PC (system 1).

Molecular weights (MW) of the six fractions were determined by gel chromatography over Sephadex G-75 (61 × 1.8 cm). The corresponding elution volumes were Ve = 66 mL (I, MW 1130), 70 (II, MW 504), 73.2 (III, IV, MW 342), and 77 (V, VI, MW 180).

Fructose (MW 180, Ve 77 mL), raffinose (MW 342, Ve 73.2 mL), and inulin (MW 5600, Ve 57.5 mL) were used as markers on the column. PC (system 2, developers 1 and 2) of SSA-1, SSA-2, SSA-3, and SSA-4 after hydrolysis by oxalic acid (1%) on a boiling-water bath for 30 min detected glucose and fructose in all samples. SSA-2 also contained galactose.

The residual raw material was extracted with water (1:10). The extracts were evaporated and purified using OU-B grade carbon. Protein was extracted by the Sevag method and precipitated with acetone. The yield of WSPS was 3.3 g (Table 1).

Next the raw material was extracted with a mixture of oxalic acid and ammonium oxalate (1:1, 0.5%) at 70°C. Pectin (7.9 g) was isolated from the extract after dialysis by precipitation with methanol. Then the raw material was extracted twice with NaOH solution (1:3, 10%) at room temperature. The extracts were neutralized with acetic acid, dialyzed against distilled water, evaporated in vacuo, and precipitated with methanol (1:4) to yield HC (1.32 g).

Total Acid Hydrolysis of GF, PS, and HC. Samples of GF were hydrolyzed by H_2SO_4 (0.5 N) for 4 h at 100°C. The hydrolysates were neutralized with $BaCO_3$, filtered, and evaporated to a syrup. PC (system 1, developer 1) identified fructose (main spot) and glucose (weak spot).

Samples of PS and HC were hydrolyzed by H_2SO_4 (2 N) at 100°C for 48 h. The hydrolysates were worked up as described above to afford glucose, galactose, rhamnose, arabinose, and xylose. Galacturonic acid was also found in the PS (system 2, developer 2).

Production of Galacturonan. Pectin (5 g, sample 7, Table 1) was heated for 4 h in H_2SO_4 (250 mL, 2 N) on a boiling-water bath. The precipitate was isolated by centrifugation, washed with H_2SO_4 (1%) and alcohol (80°), dissolved in water, dialyzed against distilled water, evaporated, and precipitated with ethanol to afford galacturonan (1.98 g), $[\alpha]_D^{20}$ +230° (*c* 0.3, 0.1 N NaOH). Total acid hydrolysis of the galacturonan and PC (system 2, developer 2) detected galacturonic acid.

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