CARBOHYDRATES OF *Alium*.

VIII. POLYSACCHARIDES OF *Allium coeruleum*

M. A. Khodzhaeva and E. S. Kondratenko*

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The group carbohydrate composition of the bulbs of *Allium coeruleum* Pall. have been studied. The qualitative and quantitative compositions of the glucofructans, pectin substances, and hemicelluloses isolated have been determined and their physicochemical characteristics are reported. A general formula for the glucofructan from *A. coeruleum* bulbs is given on the basis of the results of periodate oxidation, methylation, and IR, and ^{13}C NMR spectroscopy.

Continuing investigations of carbohydrates of plants of the family Alliceae, we have studied the polysaccharides of the bulbs of *Allium coeruleum* Pall., collected on the experimental station of VILR [All-Union Scientific Research Institute of Medicinal Plants] at the end of the vegetation period. By the successive extraction of the bulbs we isolated an ethanol-soluble fraction (31.33% of the absolutely dry weight), water-soluble polysaccharides (13.2%), pectin substances (2.5%), and hemicelluloses A (0.72%) and B (0.42%).

In the ethanol-soluble fraction, after purification with lead acetate and activated carbon, fructose, glucose, sucrose, and oligosaccharides with R_f 0.11 and 0.02 were detected by paper chromatography.

The water-soluble polysaccharides (WSPS) were freed from protein by Sevag's method and their solution was clarified by centrifugation on an ultracentrifuge. The clarified solution of WSPS was precipitated with ethanol. This gave a precipitate of WSPS-A with $[\alpha]_D^{20}$ +130°. Its molecular mass, determined by a viscometric method, was 78,000.

Its IR spectrum had absorption bands at 940, 1030, 1070, 1140, 1750, and 2940 cm^{-1}, which are characteristic for pectins [1].

In the product of complete acid hydrolysis, paper and gas–liquid chromatography showed the presence of rhamnose, xylose, arabinose, glucose, and galactose in ratio of 2.25:8:1:2.78:1.5, and also of galacturonic acid.

The methoxy group content of the WSPS-A was 7.36%.

Periodate oxidation of the WSPS-A was carried out at room temperature, the consumption of sodium periodate being 0.33 mole per 1 mole of anhydrohexose unit. On Smith degradation, paper chromatography showed the presence of arabinose, xylose, rhamnose, and galacturonic acid, and also of glycerol and erythritol.

The comparatively low consumption of sodium periodate and the presence of unoxidixed monosaccharides indicated a branched structure of the WSPS-A.

The partial hydrolysis of the WSPS-A gave a galacturonan consisting solely of galacturonic acid residues. Its IR spectrum had absorption bands at 820, 830, 945, 1020, 1110, 1340, 1405, 1635, 1750, 2935, and 3400 cm^{-1}. The pyranode form of the galacturonic acid residues was confirmed by the presence of absorption bands at 1020 and 1110 cm^{-1}. The positive specific rotation of $[\alpha]_D^{20}$ +46° indicated the α-configuration of the glycosidic bond between the galacturonic acid residues in the pyranose form.

The quantitative characteristics of the WSPS-A obtained by a titrimetric method [2] were, respectively (%): free carboxy groups, K_f — 1.35; methoxylated carboxy groups, K_e — 8.33.

*Deceased.

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The mother solution after the precipitation of the WSPS-A was evaporated and precipitated with acetone. The resulting precipitate — WSPS-B — amounted to 3.2% on the absolutely dry raw material and consisted of a white powder with $[\alpha]_D^{20}$ −40° giving no coloration with iodine.

According to the results of gel chromatography on a column of Sephadex G-75, the WSPS-B was polydisperse with molecular weights ranging from 500 to 35,000.

To obtain homogeneous GFs, fractionation was carried out with ethanol (10% solution of the WSPS in 25 ml of H_2O), and the following fractions were obtained:

Fraction	Ethanol added, ml	Yield, %	$[\alpha]_D^{20}$ (*c* 0.1; H_2O), deg	Mol. wt.
GF-I	25	4.0	—	10000—35000
GF-II	25	32.8	—30	7300
GF-III	50	41.2	—23	1800
GF-IV	—	22.0	—	500—1000

In the products of the complete acid hydrolysis of GF-II and GF-III, paper and thin-layer chromatography showed the presence of fructose and glucose, their qualitative ratios according to Kolthoff being 97.77 and 2.22% and 90.90 and 9.09%, respectively, i.e., both polysaccharides were glucofructans.

The IR spectra of GF-II and GF-III had absorption bands characteristic for glucofructans of the mixed type:

The chemical shifts in the ^{13}C NMR spectrum of GF-III also indicated that the compound was a glucofructant of the mixed type:

	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
2→1-Bound fructofuranose units	62.7	104.55	78.8	76.1	82.6	63.7
2→6-Bound fructofuranose units	62.1	105.4 104.6	78.2	76.65	81.7	64.4
α-D-Glucopyranose	93.6	72.55	74.05	70.8	73.0	61.5

Analysis of the ^{13}C NMR spectrum showed that glucose was present at the reducing end of the GF-III, as was demonstrated by the peak of the C-1 chemical shift of α-D-glucopyranose (93.6 ppm), which is characteristic for this type of bond [4].

The quantitative ratio of 2 → 1- and 2 → 6-bound fructofuranose residues and 2 → 1-attached α-D-glucopyranose residues, calculated from the integral intensities, was 8:1:1.

The periodate oxidation of the GF-II and the GF-III was carried out at room temperature, the consumptions of sodium periodate amounting to 1.10 and 1.06 moles and the amounts of formic acid liberated to 0.033 and 0.064 mole per anhydrohexose unit, respectively.

When a sample of GF-III was subjected to Smith degradation, paper chromatography showed the presence of glycerol alone.

GF-II and GF-III were methylated by Hakomori's method. The resulting permethylates of GF-II and GF-III with $[\alpha]_D^{20}$ −48° and −40° were subjected to formolysis followed by hydrolysis. The following sugars were found by thin-layer chromatography in the products of the hydrolysis of the GF-III permethylate: 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 (intense spot); and 1,3,4-tri-O-Me-D-fructose. Di-O-Me-D-fructose was detected, additionally, in a sample of the permethylate from GF-II of *A. coeruleum*.

Thus, on the basis of the results of physical and chemical methods of investigation it is possible to put forward a general formula for GF-III from the bulbs of *A. coeruleum*:

$$\beta\text{-}D\text{-Fruf-2}\rightarrow 6\text{-}\beta\text{-}D\text{-Fruf-2-}\left[\text{-1-}\beta\text{-}D\text{-Fruf-2-}\right]_8\text{-1-}\alpha\text{-}D\text{-Glcp.}$$

In the products of the acid and enzymatic hydrolyses of the PSs, the same sugars were detected by paper chromatography as in the case of the WSPS-A, but the ratio of rhamnose, cylose, arabinose, glucose, and galactose determined by GLC was 4.5:4.8:1:4:1.2.

The IR spectra of the pectin substances and of the WSPS-A were identical. The methoxy-group content in the pectin substances differed from that in the WSPS-A, amounting to 3.9%.

Hemicelluloses A and B were extracted with a 10% solution of caustic soda; after dialysis the extract deposited the HMC-A, and the mother solution was evaporated and precipitated with methanol to give the HMC-B.

The products of the acid hydrolysis of the HMC-A were shown by paper and gas–liquid chromatography to contain galactose, arabinose, xylose, and rahmnose in a ratio of 1:1.8:2.4:2.0, and in the HMC-B xylose and rhamnose were identified in a ratio of 1:1.5.

EXPERIMENTAL

Solutions were evaporated in a rotary evaporator at 40 ± 5°C. IR spectra were taken on a UR-20 instrument with KBr tablets. Specific rotations were determined on Zeiss polarimeter in a tube 1 dm long with a volume of 10 ml.

^{13}C NMR spectra were taken on a Bruker-60 instrument with a working frequency for carbon of 15.08 MHz with complete proton suppression, using 3% solutions in D_2O with methanol as internal standard. The chemical shifts are given in the δ scale.

PC was performed on FN-7,17 paper by the descending method in system 1) 1-butan-ol–pyridine–water (6:4:3), and by the ascending method in system 2) 1-propan-ol–ethyl acetate–water (7:2:1). The following reagents were used to indicate sugars: 1) aniline phthalate; 2) saturated $KMnO_4$–KIO_4–benzidine solution.

For thin-layer chromatography (TLC) we used LS 5/40 silica gel and Silufol-254 and the following solvent systems: 3) 1-butan-ol–pyridine–water (1:1:1) and 4) benzene–acetone (1:1). Revealing agents: aniline phthalate and concentrated sulfuric acid.

The gas–liquid chromatography of the samples was performed on a Tsvet-101 instrument with a flame-ionization detector under the following conditions: steel column (200 × 0.3 cm) with 5% of Silicone XE-60 on Chromaton AW (0.200-0.250 mesh), 210°C, the carrier gas being helium at the rate of 40 ml/min.

Isolation of the Polysaccharides. After inactivation with 96% ethanol, 67 g of comminuted raw material was extracted with 80% ethanol at the boiling point for 2 h four times. The yield of ES amounted to 21.5 g. The residue of the raw material was treated with water, and the crude extract was freed from protein, clarified on an ultracentrifuge (20,000 rpm, 30 min), and precipitated with ethanol in a ratio of 1:4.

The precipitate of WSPS-A obtained (6.5 g, i.e., 10% on the absolutely dry raw material) consisted of a white fibrous polysaccharide with $[\alpha]_D^{20}$ +130° (*c* 0.5; H_2O).

The supernatant solution was evaporated and precipitated with acetone to give 2.1 g of WSPS-B – 3.9% on the absolutely dry raw material. The WSPS-B formed a white power with $[\alpha]_D^{20}$ –40° (*c* 0.1; H_2O).

The raw material was then extracted with a mixture of 0.5% oxalic acid and ammonium acetate. After dialysis against distilled water the extract was evaporated to a syrup, and the syrup was precipitated with methanol in a ratio of 1:2. This gave a deposit (1.61 g) of pectin substances (PSs) in the form of a cream-colored fibrous amorphous powder with $[\alpha]_D^{20}$ +138° (*c* 0.1; H_2O).

From the remains of the raw material, a 10% solution of caustic soda extracted hemicelluloses A and B. After dialysis the HMC-A (0.47 g) deposited; the precipitate was centrifuged off and the mother solution was then evaporated and precipitated with methanol giving the HMC-B (0.27 g).

The hydrolysis of the WSPS-A, the PSs, the HMC-A, and the HMC-B was carried out with 2 N H_2SO_4 in sealed tubes at 100°C for 48 h. The hydrolysates were treated with barium carbonate and evaporated to the state of a viscous syrup. Part of the hydrolysis products was analyzed by paper chromatography (system 1) and another part was used for obtaining aldonitrile acetates for GLC [5].

Periodate Oxidation of Samples of WSPS-A. A solution of 117 mg of the WSPS-A in 30 ml of 0.05 M sodium periodate solution was left in the dark at room temperature. Aliquots with a volume of 1 ml were taken and the excess of sodium periodate was titrated with a 0.01 N solution of sodium thiosulfate. The consumption of sodium periodate in 30 days amounted to 0.33 mole and did not change further. After the addition of 1 ml of ethylene glycol and dialysis, the oxidation product was reduced with sodium tetrahydroborate, and the resulting polyalcohol was hydrolyzed with 3 ml of 1 N H_2SO_4 at 100°C for 8 h. PC showed the presence

of arabinose, xylose, rhamnose, and galacturonic acid (system 1), and also of glycerol and erythritol (system 2).

The enzymatic hydrolysis of samples of the PSs was carried out with Fluka pectinase (Switzerland) in an acid medium (pH 4) at 37°C for 48 h. The enzyme was inactivated at 100°C for 5 min. The hydrolysis products were analyzed by PC (system 1).

Partial Hydrolysis of the PSs. A solution of 1.0 g of the PSs in 50 ml of water was treated with 50 ml of 4 N H_2SO_4, and the mixture was heated in the boiling water bath for 4 h. The precipitate was separated off by centrifugation and was washed repeatedly with 1% sulfuric acid, 70% methanol, and acetone, and was then dissolved in water and the solution was dialyzed. The dialyzate was concentrated and precipitated with acetone. The yield of galacturonan was 0.35 g $[\alpha]_D^{20}$ +146° (*c* 0.25 ; 0.1 N NaOH).

Fractionation of the WSPS-B. To 25 ml of a 10% solution of the WSPS-B were added, successively, 25 ml, 25 ml, and 50 ml of ethanol, giving four fractions, GF-I to GF-IV. The fractions were separated off by centrifugation and were washed with acetone.

Determination of Molecular Weights. Samples weighing 10 mg each in 2 ml of distilled water of WSPS-B, GF-II, GF-III, dextrans with molecular weights of 10,000, 15,000, and 40,000, and raffinose were deposited on a column of Sephadex G-75 (61 × 1.8 cm). Eluates were collected in 3-ml fractions and these were analyzed by the phenol–sulfuric acid method. The WSPS-B was polydisperse (molecular weights from 500 to 35,000), while the molecular weights of the GF-II (V_e = 54.5 ml) and GF-III (V_e = 63.3 ml), determined from a graph of dependence on elution volume were 7300 and 1800, respectively.

Acid Hydrolysis of Samples of GF-II and GF-III. Samples (0.1 g) were hydrolyzed in 10 ml of 0.5 N H_2SO_4 at 100°C for 2 h. The hydrolysis products were analyzed, after neutralization with calcium carbonate, by PC (system 1) and TLC (system 3) and by Kolthoff's method. The amounts of fructose and glucose in the GF-II and GF-III were 97.77 and 2.22%, and 90.90 and 9.09%, respectively.

The periodate oxidation of the GF-II and GF-III was carried out in 30 ml of a 0.05 M solution of sodium periodate. In 6 days the consumptions of periodate were 1.10 and 1.06 moles, respectively. Glycerol was detected by PC in the product of Smith degradation (system 2).

Methylation of GF-II and GF-III. One of these glucofurans (0.5 g) was dissolved in 25 ml of dimethyl sulfoxide with stirring in a current of nitrogen (1.5-2 h), after which 10 ml of freshly prepared methylsulfinyl carbanion was added. Then 2 ml of freshly distilled methyl iodide was added dropwise to the reaction mixture, and after 1 h it was neutralized with acetic acid and treated with 200 ml of chloroform, and the organic layer was washed repeatedly with water to neutrality. The chloroform solution was evaporated to dryness and the residue was remethylated. The completeness of methylation was determined with the aid of IR spectroscopy. Permethylates of GF-II (0.33 g) and GF-III (0.31 g) were obtained. Samples (50 mg) of GF-II and GF-III were subjected to formolysis in 2.5 ml of 90% formic acid at 80°C for 1 h and, after evaporation with the addition of methanol, the residues were hydrolyzed with 0.5 N H_2SO_4 at 100°C for 6 h.

The hydrolysates were neutralized with sodium carbonate, and in the concentrated filtrate TLC (system 1) in comparison with authentic samples showed the presence of 1,3,4,6-tetra-O-methyl-D-fructose, 2,3,4,6-tetra-O-methyl-D-glucose, 3,4,6-tri-O-methyl-D-fructose, and 1,3,4-tri-O-methyl-D-fructose.

A hydrolysate of the permethylate of GF-II contained a di-O-methyl-D-fructose.

SUMMARY

The group carbohydrate composition of the bulbs of *Allium coeruleum* Pall. has been studied. The qualitative and quantitative compositions of the glucofructans, pectin substances, and hemicelluloses isolated has been determined. Physicocehmical characteristics are given.

A general formula of the glucofructant from the bulbs of *A. coeruleum* has been put forward on the basis of periodate oxidation, methylation, and IR and ^{13}C NMR spectroscopy.

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POLYSACCHARIDES FROM THE WASTES OF SOME FRUIT AND BERRY, VEGETABLE, AND TECHNICAL CROPS

D. A. Rakhimov, N. P. Yuldasheva, S. A. Khamidkhozhaev, and E. S. Kondratenko*

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The fractional isolation of water-soluble polysaccharides and pectin substances has been carried out from the wastes of fruit and berry and vegetable crops: apples, quinces, grapes, and tomatoes, and also the valves of cotton bolls. The qualitative and quantitative monosaccharide compositions of the carbohydrates isolated have been determined, and the characteristics of the pectin substances are given.

In the processing of fruit and berry and vegetable crops in the preserving factories of Uzbekistan – apples (*Malus domestica, M. pumila*), quinces (*Cydonia oblonga*), grapes (*Vitis vinifera*), and tomatoes (*Lycopersicum esculentum*) – a large amount of wastes is formed, and during the working of the heap cleaners, the valves of the bolls of the cotton plant *Gossypium hirsutim*) accumulate.

One of the possible methods for their utilization is the isolation from them of valuable polymers and, in particular, pectin substances. Interest is shown in them in pharmacy as stabilizers for suspensions and emulsifying agents [1, 2] and in the food industry as gel-forming agents [3].

We successively extracted the polysaccharides from one sample of air-dry raw material: first the water-soluble polysaccharide (WSPSs) and then the pectin substances (PSs). To determine their monosaccharide compositions, samples of the polysaccharide fractions were subjected to complete acid hydrolysis. The hydrolysates were analyzed by the PC and GLC methods. The results obtained are given in Table 1.

As can be seen from Table 1, the amounts of WSPSs in the sample investigated ranged between 1 and 4.2%. The polysaccharides isolated consisted of white powders with a creamy tinge readily soluble in water. Rhamnose, arabinose, xylose, mannose, glucose, and galactose were detected in the hydrolysis products. The pectin substances, which were obtained with yields of 1.2-15.2%, were characterized in greater detail. The pectins did not contain starch, as was shown by the negative reaction with iodine. The PSs obtained consisted of odorless white powders with a creamy tinge readily soluble in water and precipitated from their aqueous solutions by aluminum sulfate.

In the products of the complete acid hydrolysis of the PSs, in addition to the monosaccharides given in Table 1, we found a considerable amount of galacturonic acid, which was identified by PC and electrophoresis with markers. The quantitative characteristics obtained by the titrimetric method [4] are given below (%):

*Deceased.

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