*Allium* CARBOHYDRATES

# I. ISOLATION AND CHARACTERIZATION OF THE POLYSACCHARIDES

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Plants of the genus *Allium* (family Alliaceae) [1] are widely distributed throughout the terrestrial globe. In the "Flora of the USSR" 228 species are described [2]. The carbohydrates of the Central Asian species of plants of this genus have scarcely been studied.

We have investigated the polysaccharides of the bulbs of 15 species of plants of the genus *Allium*. The dried and comminuted raw material, after treatment with 96% ethanol to eliminate the ballast ethanol-soluble substances, was extracted with 82% ethanol. The combined ethanolic extracts, after purification and concentration, were subjected to analysis by paper chromatography (PC, system 1). Glucose, fructose, sucrose, and oligosaccharides with $R_f$ values less than that of sucrose were found in all the samples. Extraction of the residue of the raw material with water gave the water-soluble polysaccharides (WSPSs). As can be seen from Table 1, the amount of WSPSs in the various species of *Allium* varies within wide limits (from 4 to 76%).

The WSPSs isolated consisted of hygroscopic colorless or light cream-colored powders. They dissolved readily in water, forming sticky yellow solutions which gave no coloration with a 0.1 N solution of iodine. On complete acid hydrolysis, samples of the WSPSs formed glucose and fructose, identified by PC (system 1) and GLC, in various ratios.

It was shown by gel chromatography on Sephadex G-75 [3] that the WSPSs of the species of *Allium* described above were polydisperse and their weight-average molecular weights [4] varied from 500 to 50,000; an example is the glucofructan (GF) from *A. sativum* (Fig. 1a).

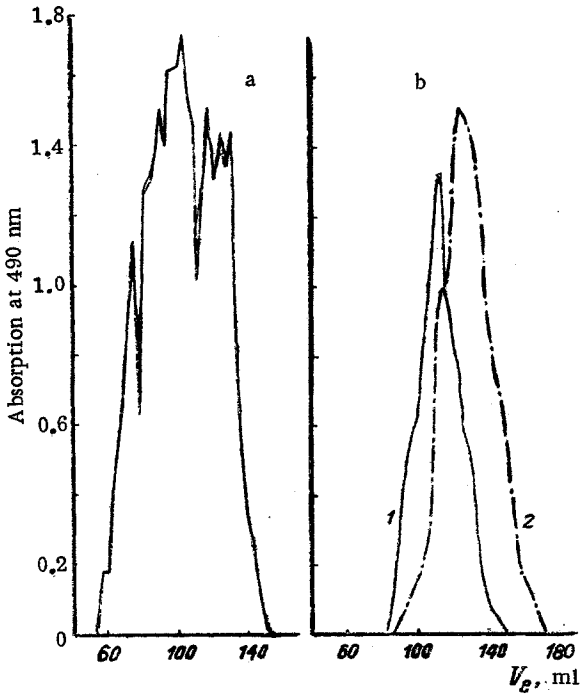


Fig. 1. Gel chromatography of the glucofructan from *A. sativum* on Sephadex G-75 (a) and of fractions II (1) and IV (2) of the glucofructan on Sephadex G-50 (b).

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TABLE 1. Amounts of WSPSs in the Bulbs of *Allium* L.

| Species of plant | Site and date of collection | Phase of development | Amount of WSPSs, % on the weight of the air-dry material |
|---|---|---|---|
| *A. atrosanguineum Schrenk* | KirgSSR, valley of the R. Tyup, May 23, 1976 | Incipient fruit-bearing | 6.0 |
| *A. aflatunense B. Fedtsch.* | Fergana oblast, Baubashata, May 28, 1975 | Flowering | 36.0 |
| *A. cepa L.* | Tashkent oblast, May 20, 1976 | Vegetation | 3.7 |
| *A. coeruleum Pall.* | KazSSR, VILR* experimental station, November 10, 1975 | End of flowering | 13.9 |
| *A. fetissowii Regel* | KazSSR, experimental station, November 10, 1975 | End of flowering | 13.4 |
| *A. giganteum Regel* | Turkmen SSR, Firyuza region, June 7, 1975 | End of fruit-bearing | 60.0 |
| *A. karelinii Poljak.* | KirgSSR, valley of the R. Tyup, May 23, 1975 | Flowering | 23.0 |
| | KazSSR, Dzhungarian Ala-Tau, June 25, 1977 | Flowering | 32.1 |
| *A. longicuspis Regel* | Tashkent oblast, valley of the R. Pskem, October 14, 1976 | Dormant state | 76.7 |
| *A. oreophilium C. A. Mey* | KirgSSR, Talasskii range, July 13, 1977 | Incipient fruit-bearing | 22.0 |
| *A. polyphyllum Kar. et. Kir.* | KirgSSR, Alai range, August 7, 1977 | Budding period | 30.0 |
| | KirgSSR, July 12, 1977 | Flowering | 14.5 |
| *A. sativum L.* | Tashkent oblast, May 20, 1976 | Vegetation | 51.0 |
| *A. sphaero-cephalum L.* | KazSSR, VILR* experimental station, October 10, 1975 | End of flowering | 28.0 |
| *A. suworowii Regel* | TadzhSSR, Western Hissar range, August 25, 1973. | Dormant state | 34.0 |
| | Dzhizak oblast, Turkestan range, May 31, 1976 | Flowering period | 35.3 |
| *A. stipitatum Regel* | Surkhandar'ya oblast, Hissar range, May 5, 1976 | Budding period | 61.0 |
| *A. turkestanicum Regel* | KazSSR, Dzhambul oblast, May 28, 1978 | Flowering | 48.5 |

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The WSPSs isolated from the bulbs of *A. sativum* were studied in more detail, since the presence in them of a glucofructan has been reported in the literature [5], but its characteristics were not given. On fractionation with ethanol [6], four fractions were obtained and their characteristics are given below:

| Fraction | Ethanol added, ml | Yield, % | $[\alpha]_D^{22}$, deg | $[\eta]_{rel}^{20}$ |
|---|---|---|---|---|
| I | 100 | 20 | — | — |
| II | 150 | 18 | —46 (*c*, 1,0; $H_2O$) | 1,14 (*c* 1,0; $H_2O$) |
| III | 350 | 19 | — | — |
| IV | 750 | 6 | —40 (*c*, 1,0; $H_2O$) | 1,06 (*c* 1,0; $H_2O$) |

The weight-average molecular weights of fractions II and IV determined from a calibration curve [4] plotted from the results for inulin and raffinose were 5000 and 1900 (Fig. 1b).

The IR spectra of fractions II and IV have absorption bands at 820, 860, and 940 $cm^{-1}$, which are characteristic for a 2 → 1 bond [7-9] and are similar to the absorption bands of inulin. Thus, from the assignment of the absorption bands the IR spectra of fractions II and

IV of the polysaccharide from *A. sativum* they can be included under glucofructans (GFs) of the inulin type. This hypothesis has also been confirmed by the results of periodate oxidation. The ease of acid hydrolysis and the negative specific rotation of the glucofructans permits the conclusion that β-glycosidic bonds between the fructofuranose residues predominate.

The periodate oxidation of fractions II and IV of the glucofructan from *A. sativum* was carried out at room temperature (the consumption of $NaIO_4$ and the amount of HCOOH produced are given in moles per mole of anhydrohexose unit):

| Fraction | Time, h | Consumption of $NaIO_4$ | HCOOH produced |
|---|---|---|---|
| II | 93 | 0.7 | 0.0518 |
| | 117 | 0.9 | 0.066 |
| | 125 | 1.03 | 0.074 |
| IV | 117 | 1.04 | 0.090 |
| Inulin | 93 | 0.94 | 0.064 |
| | 117 | 1.05 | 0.078 |

As can be seen from the figures given, in fractions II and IV of the GFs from *A. sativum* and in inulin the consumption of sodium periodate is 1 mole per mole of anhydrohexose unit.

On Smith degradation [10], glycerol and fructose were found in all the samples by PC (system 1) and GLC. When inulin was oxidized with periodic acid, Rominskii determined the amount of formic acid formed and recorded the absence of reducing sugars. However, as our experiments have shown, among the products of the Smith degradation of fractions II and IV glycerol predominates and fructose is also present, which shows the existence of a 2 → 1 bond between the fructofuranose residues with branching at $C_3$ of $C_4$ of the fructose.

When the residue of the raw material was extracted with a mixture of 0.5% solutions of oxalic acid and ammonium oxalate [12], *A. cera* and *A. sativum* yielded pectin substances (PSs). A hydrolysate of the PSs from *A. cera* was found by PC (system 1) to contain galactose, arabinose, xylose, rhamnose, and galacturonic acid, while that from *A. sativum* contained galactose, glucose, arabinose, and galacturonic acid. The gel chromatography on Sephadex G-75 of the above-described substances showed their polydispersity.

## EXPERIMENTAL

The solutions were evaporated in a rotary evaporator at 40±5°C. IR spectra were taken on a UR-20 instrument in tablets with KBr.

Paper chromatography (PC) was performed on FN-7, 17, and 13 papers by the descending method (1) in butan-1-ol — pyridine — water (6:4:3) and by the ascending method (2) in propan-1-ol — ethyl acetate — water (7:2:1) using the following reagents for revealing the spots: 1) aniline hydrogen phthalate, and 2) Bromophenol Blue.

The GLC of the samples was performed on a Tsvet-101 instrument with a flame-ionization detector using a steel column (0.3 × 200 cm) containing Chromaton N-AW DMCS (0.160-0.200 mm) impregnated with 5% of Silicone SE-30 at 180-220°C with nitrogen as the carrier gas (40 ml/min). The samples were used in the form of the trimethylsilyl derivative [13]. The inulin used was of "pure" grade, MRTU [Interrepublican Technical Specification] 6-09-2495-65, produced by the Shostka chemical reagents factory.

Isolation of the Polysaccharides. The raw material was ground, passed through a 0.25-mm sieve, and subjected to treatment with 96% ethanol on the boiling water bath for 1 hour. The residue of the raw material was treated with 82% ethanol (1:20) at 70° for one hour four times. The solutions were combined, evaporated to half volume, and filtered through a double paper filter, and the residue was washed with ethanol. The filtrate was treated with a saturated solution of lead acetate to complete the formation of the precipitate, and the excess of lead was eliminated with a saturated solution of sodium sulfate. After filtration, the filtrate was treated twice with chloroform and was concentrated. The syrup was analyzed by PC (system 1). Fructose, glucose, sucrose, and oligosaccharides with $R_f$ values smaller than that of sucrose were detected.

The remaining raw material was extracted with water (1:20) four times for two hours each at room temperature with constant stirring. The combined aqueous extracts were evaporated to half volume and were freed from protein by Sevag's method [14]. After the solvents had been

distilled off, the glucofructans were precipitated with acetone. The precipitate was washed with acetone and with ether and was dried in vacuum over $P_2O_5$.

Hydrolysis of the Glucofructans. A mixture of 50 mg of a polysaccharide and 5 ml of 0.5% $H_2SO_4$ was heated on the boiling water bath for two hours. The hydrolysate was neutralized with barium carbonate treated with KU-4 cation-exchange resin, concentrated in vacuum, chromatographed on FN-17 paper (system 1), and subjected to GLC.

Isolation of the Pectin Substances. The residue of raw material after extraction with water was treated with a mixture of equal volumes of 0.5% solutions of oxalic acid and ammonium oxalate (1:30) at 70°C for an hour four times. The combined extracts were dialyzed against distilled water and evaporated. The thick syrup was precipitated in methanol (1:3), and the precipitate was washed with methanol, acetone, and ether, and dried over $P_2O_5$. The amount of PSs in *A. cera* was 2.2 and in *A. sativum* 0.3%.

Hydrolysis of the PSs. The PSs (50 mg) were subjected to hydrolysis (2 N $H_2SO_4$, 72 h, 100°C) in a sealed tube. The hydrolysates were neutralized with $BaCO_3$, and the filtrates were treated with KU-2 cation-exchange resin, evaporated to the state of a syrup, and chromatographed (system 1) on FN-13 with an exposure time of 36 h. Galactose, arabinose, xylose, rhamnose, and galacturonic acid were identified in the PSs of *A. cera*, and galactose, glucose, arabinose, and galacturonic acid in the PSs of *A. sativum*.

Gel chromatography. Samples of the glucofructans from *A. sativum* and of raffinose and inulin (20 mg each in 2 ml of 0.3% sodium chloride solution) were deposited on a column (2.2 × 75 cm) of Sephadex G-50, and samples of dextran, inulan, and the glucofructans were deposited on a column (1.8 × 63 cm) of Sephadex G-75. They were eluted with the same solution.

The column of G-50 was calibrated by the passage of inulin (mol. wt. 5600, $V_e$ = 112.5 ml) and raffinose (mol. wt. 504, $V_e$ = 149.5 ml). The column of G-75 was calibrated by the passage of dextrans with mol. wt. 40,000, $V_e$=56 ml, and mol. wt. 20,000, $V_e$ = 74 ml, and of inulin with mol. wt. 5600, $V_e$ = 111.5 ml. Eluates were collected at the rate of 3 ml every 15 min and were analyzed by the phenol — sulfuric acid method [15]. The molecular weights of fractions II ($V_e$ = 114.5 ml) and IV ($V_e$ = 126.5 ml), determined on the G-50 with reference to inulin and raffinose, were 5000 and 1900, respectively.

Periodate Oxidation and Smith Degradation. A solution of 30 mg of the material in 1 ml of water was treated with 20 ml of 0.1 M $NaIO_4$. The consumption of sodium periodate was determined by titration with 0.01 N $NaS_2O_3$, and the amount of formic acid produced was determined by titration with 0.01 N NaOH. The polyaldehyde obtained was dialyzed against distilled water and was reduced with sodium tetrahydroborate. The polyalcohol was treated with KU-2 cation-exchange resin and was hydrolyzed with 0.5% HCl at 60°C for two hours. Glycerol and fructose were found in the hydrolyzate by TC (system 2, FN-7) and by GLC.

## SUMMARY

1. The amounts of glucofructans in 15 species of plants of the genus *Allium L.* growing in Central Asia have been studied.

2. The polydispersity of all the glucofructans isolated has been established. Fractionation of the glucofructan from *A. sativum* L. yielded homogeneous fractions with mol. wt. 1900 and 5000. On the basis of their IR spectra and the results of periodate oxidation, they have been assigned to the glucofructans of the inulin type.

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# MICROPREPARATIVE GAS—LIQUID CHROMATOGRAPHY OF THE PRODUCTS OF THE PARTIAL METHYLATION OF METHYL α-L-RHAMNOPYRANOSIDE

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In a preceding paper [1] we showed the applicability of preparative gas—liquid chromatography (GLC) to the production of the methyl ethers of methyl β-D-xylopyranoside. In the present paper we give the properties and describe the conditions for the production of individual acetates of methyl ethers of methyl α-L-rhamnopyranoside by preparative gas—liquid chromatography of the products of the partial methylation of methyl α-L-rhamnopyranoside.

Under analytical conditions in columns of QF-1 and butanediol succinate (BDS) a satisfactory separation of the acetates of the methyl ethers of methyl α-L-rhamnopyranoside is achieved. The results of preparative separation with the aid of GLC and the analytical figures for the methyl ethers obtained are given in Table 1. It must be mentioned that on the QF-1 column incomplete separation of the 2,3- and 2,4-di-O-methyl ethers is observed, and on the BDS column the 3- and 4-O-methyl ethers are poorly separated and they issue as one peak from a preparative BDS column. Consequently, the QF-1 column was used as an auxiliary column only for the separation of the 3- and 4-O-methyl ethers. The load on a two-meter column containing 10% of BDS (column A) did not exceed 300 mg of the mixture, while when a mixture of the acetates of the methyl ethers of methyl β-D-xylopyranoside were used overloading of the column set in when more than 600 mg of the mixture was applied [1]. The use of a three-meter column with 10% of BDS (column B) led to the partial separation of the 3- and 4-O-methyl ethers but not sufficiently to enable these components to be collected in the pure state. The efficiency of column B was 230 plates, while the efficiency of column A was 150 plates. At the same time, even the increase in the length of the column to 3 m (column B) did not permit the load to be increased to more than 400 mg without a serious loss of efficiency. Consequently, to obtain the 3- and 4-O-methyl ethers of methyl α-L-rhamnopyranoside we used two methods. The first method consisted in subjecting to periodate oxidation the product of the partial methylation by Purdie's method of methyl α-L-rhamnospyranoside with the highest content of 3-O-methyl ether (26%). All the components of the mixture obtained were separated well by preparative GLC on a BDS column.

The second method consisted in the use of a mixture of the 3- and 4-O-methyl ethers of the rhamnoside obtained by means of preparative GLC on a BDS column for preparative GLC on a QF-1 column. Complete separation of the 3- and 4-O-methyl ethers was observed with a load on the column of 100 mg of the mixture.

The methylation of methyl α-L-rhamnopyranoside by Kuhn's method with barium oxide in the final stages of the reaction led to an accumulation of the 2,3-di-O-methyl ether in the reaction mixture, and after methylation for 40 min the reaction mixture contained mainly the 2,3-di-O-methyl ether and the permethylated rhamnoside. This enabled the load on column B to be increased to 1.0 g of this mixture.

Analytical GLC demonstrated the chromatographic purity of all the methyl ethers separated. The results of elementary and functional analysis correspond to the theoretical figures. The total degree of extraction and the coefficient of chromatographic extraction were approximately 70 and 60%, respectively.

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