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CARBOHYDRATES OF *Allium*.

IV. GLUCOFRUCTANS OF *Allium longicuspis*

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Water-soluble polysaccharides have been isolated from the bulbs of *Allium longicuspis* Rgl. By fractionating the combined water-soluble polysaccharides, homogeneous polysaccharides with molecular weights of 15,500 and 5100 have been isolated. It has been shown by spectral and chemical methods that they consist of glucofructans of a mixed type containing glycosidic bonds of the inulin (2 → 1) and levan (2 → 6) types in a ratio of 2.1:1.

Continuing investigations of the carbohydrates of plants of the family *Alliaceae* [1], we have studied the polysaccharides of *Allium longicuspis* Regel collected in the region of the R. Pskem (Uzbed SSR).

The communited raw material that had been treated with 96% ethanol to eliminate the ballast ethanol-soluble substances was extracted with 82% ethanol. Analysis of the combined ethanolic extracts (8.9% of the absolutely dry raw material) after purification and concentrated by paper chromatography (PC, system 1) showed the presence of glucose, fructose, sucrose, and fructooligosaccharides.

Subsequent extraction of the raw material with water gave water-soluble polysaccharides (WSPSs, 76.6% of the absolutely dry raw material). The isolated WSPSs consisted of a white hygroscopic powder readily soluble in water.

Gel chromatography on Sephadex G-75 showed that the WSPSs consisted of a polydisperse polymer the molecular weight of which ranged from 2000 to 40,000. To obtain a homogeneous fraction the initial WSPSs were fractionated by precipitation from water with ethanol, the results of this procedure being given below:

Fraction	Ethanol added †, ml	Yield, % total	Molecular weight	$[\alpha]_D^{22}$, deg
GF-I	1080	3.6	20,000—40,000	—
GF-II	1000	48.1	15500	−41(H_2O,1.0)
GF-III	1000	10.0	6,000—11,000	—
GF-IV	1900	5.1	5 00	−38(H_2O,1.0)
GF-V	—	15.0	2000—3500	—

On gel chromatography, fractions GF-II and GF-IV proved to homogeneous, and their IR spectra had absorption bands at 835, 870, and 945 cm^{-1} that are characteristic for glucofructans of the mixed type [2, 8].

In the products of the complete acid hydrolysis of GF-II and GF-IV, fructose and traces of glucose were identified by PC.

*Deceased.

†50 g of WSPSs in 500 ml of water.

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By Kolthoff's method [3], 98.9% and 96.8% of fructose were found in GF-II and GF-IV, respectively.

The negative specific rotations, the rate of acid hydrolysis, and the features of the IR spectra of FG-II and GF-IV showed the presence of a β-glycosidic bond between the fructofuranose residues in these glucofructans.

The chemical shifts of the carbon atoms in the ^{13}C NMR spectrum of GF-II also confirmed the presence of linkage by 2 → 1β and 2 → 6β bonds in a ratio of 2.1:1 [4]:

	C-1	C-2	C-3	C-4	C-5	C-6
Residues of 2→1β-bound fructofuranose	62.15	104.6 104.5	78.3	76.0 76.6 *	82.5	64.0 62.4
Residues of 2→6β-bound fructofuranose units	61.7	105.15 105.0 105.3	78.45	76.3 76.7 *	81.55	64.5 62.15
Residues of α-D-glucopyranose	93.5	72.45	74.0	70.65	73.0	61.7

It follows from the figures given that glucose was present at C-2 of the reducing end of the inulin moiety of the glucofructan molecule.

The chemical shifts of the C-2 and C-4 atoms are the signals of units linked end-to-end, i.e., when the inulin and levan parts of the glucofructan are adjacent. The C-4 signal of the inulin moiety of the GF-II molecule also had a chemical shift of 76.6 ppm, this signal probably corresponding to a point of branching at C-4 to which a fructofuranose residue is attached.

Periodate oxidation with a 0.05 M solution of sodium periodate was carried out at room temperature (the consumption of $NaIO_4$ is given in moles/mole of anhydrohexose unit):

	Time, h	Consumption of $NaIO_4$	HCOOH Isalated
GF-II	99	0.98	0.049
GF-IV	99	1.0	0.073

In the products of Smith degradation, PC (system 2) showed the presence of glycerol and traces of fructose, which indicates the possibility of linkage both by 2 → 1- and 2 → 6-bonds between the fructofuranose residues with a small degree of branching at C-4 of the fructofuranose residues.

The methylation of GF-II by Hakomori's method [5] gave a permethylate with $[\alpha]_D^{22} - 46°$. In the products of formolysis followed by hydrolysis the following were identified by TLC (conditions A and B) in comparison with markers: 2,3,4,6-tetra-O-methyl-D-glucose, 1,3,4,6-tetra-O-methyl-D-fructose, 1,3,4-tri-O-methyl-D-fructose, 3,4,6-tri-O-methyl-D-fructose, and a di-O-methyl-D-fructose.

It follows from the results of analysis of the methylation products that GF-II consisted to 2 → 1β- and 2 → 6β-bound fructofuranose units with a nonreducing terminal glucopyranose residue, the point of branching being a fructofuranose residue.

The partial acid hydrolysis of GF-II from *A. longicuspis* gave a mixture of oligosaccharides which appeared on a paper chromatogram with R_{fFru} 0.99, 0.80, 0.70, 0.54, 0.47, and 0.12, also with a spot remaining at the start.

The combined oligosaccharides were separated preparatively on a column of Sephadex G-50, and oligosaccharide fractions (OFs) were obtained. Fraction 1 had a molecular weight of 180 and $[\alpha]_D^{22} - 92°$ (c 1.0; H_2O), and it was identified as fructose.

When the fractions were purified by repeated chromatography on a column of Sephadex G-25, compounds with the following characteristics were obtained:

	OF-1	OF-2	OF-3	OF-4	OF-5	OF-6	OF-7	OF-8
Mol. wt.	180	342	342	504	666	1400	3600	9000
Monomeric composition	Fru	Fru	Fru Glc	Fru	Fru	Fru	Fru Glc	Fru Glc
$[\alpha]_D^{22}$, deg	−92	−37.6	+66.5	—	—	—	−35	−39

*For end-to-end linked units 76.7 ppm.

The homogeneity of the fructose and of fractions 2-5 was determined by TLC on silica gel (conditions C) [6].

When OF-3 was subjected to complete hydrolysis, the formation of fructose and glucose was shown by PC. The spots of the sugar had equal intensities and quantitative determination by Kolthoff's method gave 50% of fructose and 50% of glucose.

Hakomori's methylation [5] gave the permethylate of OF-3, which had $[\alpha]_D^{22}$ – 69.3° (c 1.0; CH_3OH). In a hydrolysate of the permethylate of OF-3, 2,3,4,6-tetra-O-methyl-D-glucose and 1,3,4,6-tetra-O-methyl-D-fructose were identified by TLC (conditions A and B), the spots of the two sugars again having equal intensities.

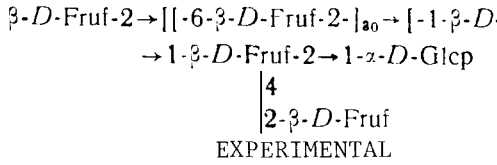
Thus, OF-3 was sucrose. OF-7 and OF-8 were, according to their molecular weights, polysaccharides and were characterized by the ^{13}C NMR spectroscopic methods.

Below we give the chemical shifts of the carbon atoms in the ^{13}C NMR spectra of the oligosaccharides OF-7 and OF-8 (ppm):

	C-1	C-2	C-3	C-4	C-5	C-6
Residues of 2→1β-bound fructofuranose units						
OF-7	62.2	104.35	78.3	76.0	82.3	63.5
OF-8	62.15	104.4	78.2	76.0	82.5	63.5
Residues of 2→6β-bound fructofuranose units						
OF-7	61.8	104.9 105.0	78.75	76.7	81.4	64.3
OF-8	61.7	105.0 105.1	78.7 78.3	76.7	81.55	64.3
α-D-Glucopyranose residues	93.5	72.9	73.9	70.7	72.3	61.7

It followed from the characteristics of the OFs obtained that on hydrolysis of the initial GF-II the first effect was probably the cleavage of the 2 → 6 bonds, and the ratio of 2 → 1β to 2 → 6β bonds increased in OF-8 to 2.72:1 and in OF-7 to 4.2:1. In the following step of partial hydrolysis, probably, the 2 → 1 bonds were cleaved, which was confirmed by the presence of sucrose.

On the basis of the characteristics of the physicochemical properties of GF-II, the features of its IR and ^{13}C NMR spectra, and the results of periodate oxidation, methylation, and partial cleavage, the following structure may be proposed for the GF-II from the bulbs of *Allium longicuspis*:



EXPERIMENTAL

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

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

PC was performed on FN11,17 paper by the descending method with butan-1-ol—pyridine—water (6:4:3) (1) and by the ascending method with propan-1-ol—ethyl acetate (7:2:1) (2). The presence of sugars was revealed by the reagents: 1) aniline hydrogen phthalate; and 2) a saturated solution of KIO_4—$KMnO_4$—benzidine.

Thin-layer chromatography (TLC) was performed on KSK silica gel and on Silufol UV-254 plates in the solvent systems: benzene—acetone—water (5:5:1) (A); benzene—acetone (4:1) (B); and propan-1-ol—ethyl acetate—water (6:1:3) (C). The revealing agents used were concentrated sulfuric acid, resorcinol, urea, and aniline phthalate.

Isolation. The polysaccharide was isolated from the bulbs of *A. longicuspis* in a similar manner to that described in [1].

Fractionation. Four 1000-ml portions of ethanol were added successively to 50 ml of a 10% solution of the polysaccharide, giving fractions GF-I—GF-V. The fractions were separated by centrifugation and were washed with acetone.

Gel Chromatography. Samples of the glucofructans from the bulbs of *A. longicuspis*, fructose, sucrose, raffinose, inulin, and dextrans 10,000, 15,000, 20,000, 40,000, and 80,000 (20 mg each) in 2 ml of distilled water were deposited on a column of Sephadex G-75 (61 × 1.8 cm). The fractionation and purification of the combined oligosaccharides were carried out on columns of Sephadex G-25 (53 × 1.8 cm) and G-50 (55 × 3.4 cm). The eluates were collected in 3-ml portions over 15 min and were analyzed by the phenol–sulfuric acid method [7].

Hydrolysis. A mixture of 100 mg of fraction GF-II and 10 ml of 0.5% H_2SO_4 was heated on the boiling water bath for 2 h.

The hydrolysate was neutralized with calcium carbonate, treated with KU-4 cation-exchange resin (H^+), and concentrated in vacuum. The product obtained was subjected to PC (system 1) and to Kolthoff analysis.

The periodate oxidation and methylation of GF-II and GF-V were carried out as described in [8].

Partial Hydrolysis. GF-II (3.3 g) was heated in a mixture of 2.5 ml of 1 N H_2SO_4 and 150 ml of distilled water at 70°C for 20 min. The hydrolysate was neutralized with calcium carbonate, deionized with KU-4 (H^+), and subjected to PC. Fractions OF-2–5 were hydrolyzed with 1% oxalic acid on the boiling water bath for 30 min. OF-6–7 were hydrolyzed in a similar manner to the initial GF-II.

SUMMARY

The fractionation of the total water-soluble polysaccharides from the bulbs of *Allium longicuspis* Rgl. has yielded homogeneous polysaccharides with molecular weights of 15,500 and 5,100.

It has been shown by spectral and chemical methods that these polysaccharides are glucofructans of a mixed type containing glycosidic bonds of both the inulin (2 → 1) and the levan (2 → 6) types, in a ratio of 2.1:1.

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