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CARBOHYDRATES OF ALLIUM.
XIII. GLUCOFRUCTANS OF *Allium suvorovii*

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The structures of the glucofructans GFAS-B3 and GFAS-I have been established on the basis of the results of periodate oxidation, methylation, and IR and ^{13}C NMR spectroscopies. It has been shown that the glucofructans studied are compounds containing both inulin, (2→1)β, and levan, (2→6)β, glycosidic bonds.

Continuing the chemical investigation of plants of the genus *Allium* [1, 2] we have studied the glucofructans of the bulbs of *Allium suvorovii* Rgl. (GFAS) collected in the flowering phase in the village of Darkhas, Lailak region, Republic of Kyrgyzstan.

In order to obtain more homogeneous fractions of the glucofructan with lower molecular masses, they were extracted from the raw material successively with ethanol (90-30%). As can be seen from the figures given below, a large set of fractions was obtained

GFAS fractions (extractant alcohol, %)	Yield, % on initial	Mobility in PC
A (90)	3.00	
B (85)	12.24	Mobile
C (80)	9.50	
D (75)	21.50	
E (70)	18.32	
F (65)	6.64	
G (60)	2.42	
H (55)	1.56	Immobile
I (50)	0.77	
J (40)	0.88	
K (30)	0.50	

In the glucofructans GFAS-A, B, and C we detected mono- and oligosaccharides, while the remaining fractions were immobile on paper chromatography and consisted of high-molecular-mass glucofructans.

The homogeneity of the fractions obtained was confirmed by gel chromatography on columns of Sephadex G-75 and G-100. All the fractions were polydisperse, apart from fraction GFAS-H, the molecular mass (MM) of which, determined by gel filtration on a column of Sephadex G-75, proved to be 35,000. A fraction with a lower MM, GFAS-B was rechromatographed on a column of Sephadex-15, and seven fractions were obtained, the molecular masses of which were determined on a column of Sephadex G-75. The characteristics of glucofructans GFAS-B are given below:

	B_1	B_2	B_3	B_4	B_5	B_6	B_7
Yield, % of initial	10	19	40	18	7	3	Tr.
Molecular mass	4000	3500	3000	1500	100	180—1000	180
$[\alpha]_D^{22}$, deg	−47	−44	−49	−39	−34	—	—
(c 1.0; H_2O)	—	93.0	92.5	90.0	—	—	100
Fructose content, %							

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TABLE 1. Chemical Shifts in the ^{13}C NMR Spectra of GFAS-B_3 and GFAS-H from *A. Suvorovii*

Residues	C-1	C-2	C-3	C-4	C-5	C-6
(2→1-bound) fructose units of						
GFAS-H	62.0	104.47	78.2	75.9 76.7*	82.3	63.45
GFAS-B_3	62.0	104.85	78.2	75.9 76.7	82.3	63.5
(2→6-bound) fructose units of						
GFAS-H	61.7	105.15 104.85*	78.2	76.2	81.4	64.5
GFAS-B_3	61.7	105.15	78.2	76.2	81.4	64.5
α-D-Glucopyranose	93.3	72.85	73.3	70.55	72.25	61.8

*Junction.

As glucofructans GFAS-B_3 and GFAS-H were homogeneous and formed the bulk of the polysaccharides, we studied their chemical structures. In the products of the complete acid hydrolysis of the glucofructans B (systems 1 and 3, revealing agents 1 and 2), we detected fructose and glucose. The fructose contents of the glucofructans GFAS-H and GFAS-B_3, determined by Kolthoff's method [3] were 99.5 and 92.5%, respectively.

The periodate oxidation of the glucofructans was carried out at room temperature with a 0.05 M solution of $NaIO_4$:

Sample	Time, h	Consumption of $NaIO_4$, moles	HCOOH isolated, moles
GFAS-B_3	30	0.51	0.035
	54	0.60	
	120	0.90	
	140	0.98	
GFAS-H	30	0.56	0.050
	54	0.58	
	120	0.86	
	140	0.99	

In the products of the Smith degradation [4] of all the samples, glycerol was found in predominating amount by GC (systems 2 and 3; revealing agents 1 and 4) which may indicate the presence of both 2→1- and 2→6- bonds, while in samples GFAS-B_3 and GFAS-H trace amounts of fructose, showing the presence of branching in the carbohydrate chain, were detected.

The methylation of GFAS-H and GHAS-B_3 was carried out twice by Hakomori's method [5], and permethylates were obtained with yields of 80-86%, $[\alpha]_D^{20}$ −50° and −57° (*c* 1.0; $CHCl_3$), respectively.

The products of the hydrolysis of the permethylates were identified by TLC (system 4, revealing agents 2 and 3) and GLC in comparison with standards, and the following sugars were detected: 2,3,4,6-tetra-O-methyl-D-glucose, 1,3,4,6-tetra-O-methyl-D-fructose, 3,4,6-tri-O-methyl-D-fructose, and 1,3,4-tri-O-methyl-D-fructose. In addition to the above-mentioned sugars, a hydrolysate of the permethylate of GFAS-H also contained a di-O-methyl-D-hexose, which was isolated in the individual form and, after demethylation, was identified as fructose.

It is obvious from the analysis of the methylation products that the polymeric chains of GFAS-H and GFAS-B_3 contained both (2→1)β- and (2→6)β-bound fructofuranose units, while the nonreducing terminal unit in each case was α-D-glucopyranose, and GFAS-H also contained a branch at C-4 of fructofuranose, as was confirmed by the results of periodate oxidation.

We used the method of ^{13}C NMR spectroscopy [6, 7] more widely for the study of the structures of the glucofructans, pursuing two aims simultaneously: in the first place, to obtain confirmation of the results on the structure of the polymers established by chemical methods, and, in the second place, to show — which appeared to us to be useful — that this method can be employed successfully for investigating the polysaccharides of *Allium* L.

It follows from an analysis of the ^{13}C NMR spectra of glucofructans GFAS-B_3 and GFAS-H that the polysaccharides were not mechanical mixtures of inulin and levan, since there were peaks at 104.85* (C-2) and 76.7* (C-4) relating to C-2 and C-4 of abutting units of (2→1)β- and (2→6)β-bound fructofuranose units (Table 1).

Glucose was present only at the "reducing end" of the polymeric chain and was attached to C-2 of the inulin unit but not to C-6, as was shown by the chemical shift of the C-1 signal of α-D-glucopyranose of 93.3 ppm, which is characteristic for this type of bond.

In the high-molecular mass sample GFAS-H, the signals of α-D-Glc could be seen feebly, because of the decrease of its proportion with increasing molecular mass of the glucofructan.

From the integral intensities of the chemical shits we calculated the ratio of (2→1)β- and (2→6)β-bonds, which was 1.4:1 for GFAS-B_3 and for GFAS-H.

Thus, the ^{13}C NMR spectra of the glucofructans GFAS-B_3 and GFAS-H fully confirm the monosaccharide composition and the types and configurations of the glycosidic bonds established by chemical methods.

Summarizing all the facts obtained with the aid of chemical methods and ^{13}C NMR spectroscopy, it may be assumed that GFAS-B_3 and GFAS-H consisted of (2→1)β- and (2→6)β-bound fructofuranose residues with nonreducing terminal sucrose fragments, and in GFAS-H the branching sites were the C_4 atoms of the β-fructofuranose units.

EXPERIMENTAL

Solutions were evaporated in a rotary evaporator at 40 ± 5°C. Paper chromatography (PC) was conducted on Filtrak FN-7.11 paper (Germany) by the descending and ascending methods using the following solvent systems (by volume): 1) butan-1-ol-pyridine–water (6:4:3); 2) propanol–ethyl acetate–water (7:2:1); and 3) water-saturated phenol (lower layer).

Analytical TLC was performed on type TSK silica gel and on Silufol UV-254 (Chemapol) using system 4) benzene–acetone–water (5:5:1).

The following reagents were used for showing up the spots: 1) acid aniline phthlate; 2) the Bonner reagent; 3) *o*-toluidine salicylate; 4) Bromophemol Blue/boric acid.

Specific rotations were determined on a Zeiss polarimeter in a tube 1 dm long with a volume of 10 ml and a tube 0.5 dm long with a volume of 1 ml at 20 ± 3°C.

IR spectra were taken on a UR-20 instrument in tablets with KBr and paraffin wax. The gas–liquid chromatography (GLC) of the samples was carried out in a Tsvet 101 instrument with a flame-ionization detector. Steel column (0.3 × 200 cm); Chromaton N-AW-DMCS (0.160 × 0.200 mm), impregnated with 5% of Silicone XE-60, the carrier gas being nitrogen at 40 ml/min.

^{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 of proton effects. The samples were used in the form of 3% solutions in D_2O with methanol as the internal standard, its chemical shift relative to TMS being taken as 50.15 ppm. Chemical shifts are given in the δ-scale.

Gel chromatography was conducted on columns of Sephadexes G-50 and G-100. Distilled water was used as the eluent. The eluates were collected in 3 ± 0.1-ml fractions and were analyzed by the phenol/sulfuric acid method.

Weight-average molecular masses were determined from a calibration curve of the dependence of the molecular mass (MM) on the elution volume (V_e) [8].

Isolation of the Glucofructans. The comminuted raw material was extracted successively with chloroform and with alcohol at concentrations of 90-30% with 5% steps at the boiling point for 1 hour each time. Each fraction was separated by filtration and was evaporated to dryness. This gave the glucofructan fractions A-K.

Gel filtration of GFAS-H on a column of Sephadex G-100 (1.8 × 32 cm) showed MM 35,000 (V_e ±39.5 ml). GFAS-B was rechromatographed on Sephadex G-25 (2.5 × 35 cm) and seven fractions were obtained, GFAS B_1-GFAS B_7, with V_e's of 50.5, 56.9, 63.3, 69.8, 76.8, 87.3, and 91.2 ml, respectively. Fraction GFAS-B_3 had the highest yield and was homogeneous, with MM 3000.

Hydrolysis of GFAS-B_3 and GFAS-H. A sample (50 mg) was hydrolyzed with 0.5 N H_2SO_4 (2.5 ml) for 2 h in the boiling water bath. The hydrolysis products were neutralized with calcium carbonate, deionized with KU-4 (H^+), and concentrated. One part of each hydrolysate was analyzed by PC (system 1, revealing agent 1), and fructose and glucose were

detected, there being 92.5% of fructose and 7.5% of glucose in GFAS-B_3 and 99.5% of fructose and 0.5% of glucose in GFAS-H, as determined by Kolthoff's method [3].

Methylation of GFAS-B_3 and GFAS-H. In each case, a 0.4-g sample was methylated twice by Hakomori's method, the fully methylated products being obtained with yields of 0.34 and 0.36 g. The IR spectra of the permethylates lacked the absorption band of a hydroxy group (3200-3600 cm^{-1}) $[\alpha]_D^{24}$ −50° (*c* 1.0; chlf) for GFAS-H and $[\alpha]_D^{24}$ −57° (*c* 1.0; chlf) for GFAS-B_3.

Formolysis and Hydrolysis of the Permethylates of GFAS-B_3 and GFAS-H. Each permethylate (0.1 g) was heated in 5 ml of 90% HCOOH in the boiling water bath for 1 h, and the mixture was then evaporated. The dry residue was hydrolyzed in 2.5 ml of 0.5 N H_2SO_4 at 95°C for 7 h. The hydrolysates were worked up in the usual way, and TLC (system 4, revealing agents 2 and 3) showed the presence of 2,3,4,6-tetra-O-methyl-D-glucose, 1,3,4,6-tetra-O-methyl-D-fructose, 3,4,6-tri-O-methyl-D-fructose, and 1,3,4-tri-O-methyl-D-fructose. Part of each permethylate was subjected to methanolysis with a 5% methanolic solution of HCl at 65°C for 6 h. The methanolysate was neutralized with Ag_2CO_3 and, after filtration and evaporation, was analyzed by GLC.

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