CARBOHYDRATES OF *Allium*
XI. GLUCOFRUCTANS OF *Allium suvorovii*

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The structure of glucofructan GFAS-B has been established on the basis of the results of periodate and chromic oxidation, methylation, and IR and ^{13}C NMR spectroscopy.

The glucofructan from *Allium suvorovii* Rgl. is a polysaccharide containing both inulin $(2\rightarrow 1)_{\beta}$ and levan $(2\rightarrow 6)_{\beta}$ glycosidic bonds. Continuing a chemical study of plants of the genus *Allium* [1, 2], we have investigated the glucofructans of the bulbs of *A. suvorovii* gathered in the dormancy phase in the environs of the village of Sina (Western Hissar).

The ground air-dry raw material was subjected to successive extraction with 96—82% alcohol and with water. The water-soluble polysaccharide — the initial glucofructan from *A. suvorovii* (GFAS) — was fractionated on a column of activated carbon (Table 1).

Seven fractions were obtained, which were subjected to gel chromatography on Sephadex G-75 and to PC. GFAS-B and GFAS-E were homogeneous, with molecular masses of 8000 and 504, respectively.

Fructose and glucose were detected in the products of the complete acid hydrolysis of GFAS by PC (system 1). The quantitative level of fructose in GFAS-B, determined by Kolthoff's method, was 97.8%.

Periodate oxidation of GFAS-B was conducted at room temperature with 0.05 M $NaIO_4$ [3]. With a consumption of $NaIO_4$ of 0.97 mole the amount of HCOOH liberated was 0.080 mole.

In the product of Smith degradation [4], a predominating amount of glycerol was detected by PC (systems 2 and 3), which may indicate the presence of both 2→1 and 2→6 bonds, together with trace amounts of fructose, showing the presence of branching in the carbohydrate chain. In the degradation products of GFAS-B, a ratio of glycerol to fructose of 47:1 was found by GLC.

The acetylation of GFAS-B with acetic anhydride in pyridine gave a peracetate with $[\alpha]_D^{24} -475°$ (*c* 0.25; $CHCl_3$), the negative value of which presupposes a β-glycosidic bond between the fructofuranose residues of the glucofructan.

When the peracetate of GFAS-B was oxidized with chromic anhydride [5], no fructose was detected in the reaction products, which contained trace amounts of glucose. This showed the β-configuration of the glycosidic bond between the fructofuranose units and an α-glycosidic bond of the glucose.

The Hakomori methylation [6] of GFAS-B gave a permethylate with a yield of 86%, $[\alpha]_D^{20} -540°$ (*c* 1.0; $CHCl_3$).

Products of the hydrolysis of the permethylate were identified by TLC (system 4) in comparison with markers, 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, 1,3,4-tri-O-methyl-*D*-fructose, and a di-O-methyl-*D*-hexose that was isolated in the individual form and, after demethylation, was identified as 3,6-di-O-methyl-*D*-fructose.

It was obvious from the analysis of the methylation products that the polymeric chain of GFAS-B contained both 2→1- and 2→6-bound fructofuranose units, that the nonreducing terminal residue was *D*-glucopyranose, and that GFAS-B also included branching at C-4 of fructofuranose residues.

To confirm the details of the structure of GFAS-B determined by chemical methods, we used ^{13}C NMR spectroscopy [7].

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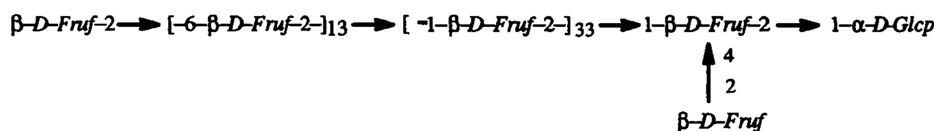
TABLE 1. Fractionation of GFAS on a Column of Activated Carbon

Fraction	Eluent	Yield, %	Mol. mass	$[\alpha]_D$, deg.
GFAS-A	H_2O	31.0	10000-50000	-
	Alcohol, %			
GFAS-B	5	7.0	8000	-26
GFAS-C	10	7.0	7000-504	-
GFAS-D	15	10.0	4000-2500	-
GFAS-E	20	10.0	504	+27
GFAS-F	25	12.0	180-342	-
GFAS-G	30	20.0	180	-

It followed from an analysis of the ^{13}C NMR spectrum of GFAS-B that the polysaccharide was not a mechanical mixture of inulin and levan, since there were peaks at 104.85 and 76.7 ppm relating, respectively, to C-2 and C-4 of abutting units of the 2→1- and 2→6-bound fructofuranose residues.

By summing all the facts obtained with the aid of chemical and physical methods, it is possible to propose the following structural formula for GFAS-B:



EXPERIMENTAL

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); 3) water-saturated phenol (lower layer).

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

We used the following spot reagents: 1) aniline hydrogen phthalate; 2) the Bonner reagent [8]; 3) Bromophenol Blue—boric acid; and 4) *o*-toluidine salicylate.

Specific rotations were determined on a Zeiss polarimeter in a tube 1 dm long with a volume of 10 ml and in one 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 in petrolatum. The gas-liquid chromatography (GLC) of samples was conducted in a Tsvet-101 instrument with a flame-ionization detector. Conditions: steel column (0.3 × 200 cm), Chromaton N-AW-DMCS (0.160 × 0.200 mm) impregnated with 5% of Silicone XE-60; 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 proton suppression. We used 3% solutions in D_2O with methanol as internal standard, its chemical shift relative to TMS being taken as 50.15 ppm; chemical shifts are given on the δ-scale (ppm).

Gel chromatography was conducted on a column of Sephadex G-50. Elution was effected with distilled water. The eluates were collected in 3±0.1-ml fractions and were analyzed by the phenol/sulfuric acid method [9]. Weight-average molecular masses were determined from a calibration curve of the dependence of the M.m. on the elution volume V_e [10].

The glucofructans were isolated as described previously [1].

The fractionation of the initial total GFASs was achieved on a column of type DAU activated wood charcoal [6]. The charcoal was first treated with 15% acetic acid and washed with distilled water to neutrality. A column (3.5 × 105 cm) was filled with the charcoal, and this was again washed with water. A sample (10 g in 20 ml of water) was deposited on the

column and eluted successively with aqueous solutions of alcohol — 5, 10, 15, 20, 25, and 30%. This gave fractions A, B, C, D, E, F, and G, respectively. The eluates were evaporated and subjected to PC (system 1, spot reagent 1) and to gel chromatography on G-75.

Samples of the glucofructans GFAS-B and GFAS-E, and of dextrans, inulin, and raffinose (20 mg each in 2 ml of a 0.3% solution of sodium chloride) were deposited on a column (1.5 × 46 cm) of Sephadex G-75. The column was calibrated by the passage of D 40000 (V_e = 44 ml), D 20000 (V_e = 50 ml), D 15000 (V_e = 65 ml), inulin—16000 (V_e = 69 ml), -10000 (V_e = 57 ml), and -5600 (V_e = 63 ml), and raffinose 504 (V_e = 86 ml). According to the calibration curve the fractions GFAS-B (V_e = 59 ml) and GFAS-E (V_e = 84 ml) had molecular masses of 8000 and 504, respectively.

Complete Acid Hydrolysis of GFAS-B and -E. Samples (0.1 g each) were hydrolyzed with 0.5 N H_2SO_4 in the boiling water bath: GFAS-E for 2 h, and GFAS-B for 4 h. The hydrolysates were neutralized with $CaCO_3$, deionized with KU-4 cation-exchanger (H^+), and evaporated to viscous syrups. Fructose and glucose were detected in them by PC (systems 1 and 3, spot reagents 1 and 2). The proportion of fructose in the GFAS-B amounted to 97.8%.

Periodate Oxidation and Smith Degradation of GFAS-B. A solution of 0.155 g of the substance in 10 ml of water was treated with 10 ml of 0.1 M $NaIO_4$ and the mixture was left at room temperature.

After every 10 h, 1-ml aliquots were removed and titrated with sodium thiosulfate solution. The HCOOH liberated was titrated with 0.01 N caustic soda and found its amount (0.08 mole). The oxidation product was dialyzed against a flow of distilled water for a day, the dialyzed polyaldehyde solution was reduced with 0.15 g of sodium tetrahydroborate and deionized with KU-4 cation-exchanger (H^+), and the filtrate was evaporated to dryness. The polyalcohol so obtained was hydrolyzed with 0.5 N H_2SO_4 (7 ml) in the boiling water bath for 4 h, neutralized with calcium carbonate, and deionized with KU-4 cation-exchanger (H^+). Glycerol and trace amounts of fructose were detected by PC (systems 2 and 3, spot reagents 1 and 3). Part of the hydrolysate was analyzed by GLC in the form of trimethylsilyl derivatives, and the ratio of glycerol and fructose was calculated (47:1).

Acetylation of GFAS-B. The substance (0.48 g) was treated with pyridine (5 ml), and the mixture was stirred at 40°C for 45 min. Then 1 ml of acetic anhydride was added and, after an hour, another 4 ml, and the reaction mixture was stirred for 8 h and was poured into ice water. The resulting precipitate was separated off, washed with water and with alcohol, and dried over P_2O_5.

The peracetate (0.49 g) had $[\alpha]_D^{24}$ -47° (*c* 0.25; $CHCl_3$) and its IR spectrum showed absorption bands at 1235 and 1750 cm^{-1}, while the absorption band of a hydroxyl group (3200—3600 cm^{-1}) was absent.

Chromic oxidation. The peracetate of fraction GFAS-B dried to constant weight (0.2 g) was oxidized with 0.6 g of CrO_3 in 7 ml of glacial acetic acid at 50°C for 4 h [5]. The oxidation product was diluted with water and extracted with chloroform, and the extract was evaporated to dryness. The dry residue was dissolved in 3 ml of 0.5 N H_2SO_4 and was hydrolyzed at 95°C for 4 h.

A weak spot of glucose was detected by PC (system 1, spot reagent 1).

Methylation of GFAS-B. A solution of 0.1 g of the substance in 2 ml of dimethylformamide was treated with 0.5 g of BaO and $Ba(OH)_2$ (in a ratio of 25:1), and, after an hour, 0.5 ml of methyl iodide was added, and the reaction mixture was stirred for two days. Then the deposit was centrifuged off, the solution was precipitated with alcohol, and the precipitate was dried and remethylated. After the second methylation the GFAS-B permethylate had $[\alpha]_D^{24}$ -54° (*c* 1.0; $CHCl_3$).

Formolysis and Hydrolysis of the Permethylate of GFAS-B. The permethylate (50 mg) in 2.5 ml of 90% formic acid was heated in the boiling water bath for one hour. Then it was cooled and evaporated. The residue was dissolved in 25 ml of 0.5 N H_2SO_4 and hydrolyzed at 100°C for 8 h. The hydrolysate of the permethylate was worked up in the usual way, and TLC 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, 1,3,4-tri-O-methyl-*D*-fructose, and a di-O-methyl-*D*-hexose. The mixture of methylated sugars from GFAS-B was subjected to preparative thin-layer chromatography (systems 2 and 4, spot reagents 2 and 4), and the zones corresponding to spots with R_f 0.53 and 0.24 were separated and eluted with chloroform. This gave 1,3,4-tri-O-methyl-*D*-fructose with $[\alpha]_D^{20}$ -4° (*c* 0.5; CH_3OH), and a di-O-methyl-*D*-hexose, which was treated with the Bonner reagent. There was no white spot on a yellow background, i.e., the substance was not oxidized, and after its demethylation fructose was detected. Consequently, the di-O -methyl-*D*-hexose was 3,6-di-O-methyl-*D*-fructose.

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