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\(\rightarrow\)1 and 2\(\rightarrow\)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 \([\alpha]_D^{24} = 475^\circ\) (c 0.25; CHCl\(_3\)), the negative value of which presupposes a \(\beta\)-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 \(\beta\)-configuration of the glycosidic bond between the fructofuranose units and an \(\alpha\)-glycosidic bond of the glucose.

The Hakomori methylation [6] of GFAS-B gave a permethylate with a yield of 86%, \([\alpha]_D^{20} = 540^\circ\) (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\(\rightarrow\)1 and 2\(\rightarrow\)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].
TABLE 1. Fractionation of GFAS on a Column of Activated Carbon

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Eluent</th>
<th>Yield, %</th>
<th>Mol. mass</th>
<th>[α]D, deg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFAS-A</td>
<td>H2O</td>
<td>31.0</td>
<td>10000-50000</td>
<td>-</td>
</tr>
<tr>
<td>GFAS-B</td>
<td>5</td>
<td>7.0</td>
<td>8000</td>
<td>-26</td>
</tr>
<tr>
<td>GFAS-C</td>
<td>10</td>
<td>7.0</td>
<td>7000-504</td>
<td>-</td>
</tr>
<tr>
<td>GFAS-D</td>
<td>15</td>
<td>10.0</td>
<td>4000-2500</td>
<td>-</td>
</tr>
<tr>
<td>GFAS-E</td>
<td>20</td>
<td>10.0</td>
<td>504</td>
<td>+27</td>
</tr>
<tr>
<td>GFAS-F</td>
<td>25</td>
<td>12.0</td>
<td>180-342</td>
<td>-</td>
</tr>
<tr>
<td>GFAS-G</td>
<td>30</td>
<td>20.0</td>
<td>180</td>
<td>-</td>
</tr>
</tbody>
</table>

It followed from an analysis of the 13C 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:

\[
\beta-D-Fnu \stackrel{2-6}{\longrightarrow} \beta-D-Fnu \stackrel{2-1}{\longrightarrow} \beta-D-Fnu \stackrel{2-1}{\longrightarrow} \alpha-D-Glc
\]

EXPERIMENTAL

Paper chromatography (PC) was conducted on Filtrak FN-711 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 x 200 cm), Chromaton N-AW-DMCS (0.160 x 0.200 mm) impregnated with 5% of Silicone XE-60; nitrogen at 40 ml/min. 13C 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 D2O 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 Ve [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