was deionized with AB-17 ion-exchange resin (OH^-), filtered from the resin, and evaporated, and the residue was acetylated with acetic anhydride (1 ml) in pyridine (1.3 ml). The acetate of the 3,6-di-O-methyl ether was chromatographed on a preparative column containing 10% of the phase NPGS at 180°C. The load on the column was 150 mg. As a result, the acetate of the 3,6-di-O-methyl ether was obtained with a yield of 75 mg, $[\alpha]_D^{20}$ +120.3° (c 0.9).

CONCLUSION

Methods are described for obtaining the acetates of mono- and di-O-methyl ethers of methyl α-D-glucopyranoside, which were isolated with the aid of preparative liquid and gas-liquid chromatography of the products of partial methylation of methyl α-D-glucopyranoside, and their properties are given.

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CARBOHYDRATES OF *Allium*.

II. A NEW TYPE OF GLUCOFRUCTAN FROM *Allium sativum*

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By fractionating the total glucofructan from the bulbs of *Allium sativum* L. a homogeneous glucofructan with a molecular weight of 2300 has been isolated. On the basis of its characteristics, a comparison of IR and ^{13}C NMR spectra, and the results of periodate and chromium trioxide oxidation and of methylation, it has been established that this glucofructan is a new type of compound of this class, containing both inulin (2 → 1) and levan (2 → 6) glycosidic bonds.

We have previously described the isolation of glucofructans from *Allium sativum* L. and their separation into four fractions [1]. Continuing these investigations, we have isolated by successive precipitation with ethanol, a homogeneous fraction IVa, intermediate between fractions III and IV, with yield of 20% of the total glucofructans (Fig. 1).

The weight-average molecular weight of fraction IVa determined by gel chromatography on Sephadex G-75 using a calibration curve plotted from the results for dextran, inulin, and raffinose was 2300 + 10%, $[\alpha]_D^{22}$ −42° (c 1.0; H_2O) and $[\eta]_{rel}^{26}$ 1.04. D-fructose and traces of D-glucose were detected by PC (conditions I) and GLC (conditions A) in the products of the complete acid hydrolysis of the fraction. The quantitative determination of D-fructose by the method of Kolthoff [2] and Bertrand [3] gave a figure of 94%.

Two types of plant glucofructans have been described in the literature — polysaccharides of the type of inulin, characterized by 2 →1 bonds between the fructose units [4-6], and those of the type of levan with 2 → 6 bonds [7]. The types of glucofructans can be distinguished by means of their characteristic absorption bands in the 800-1000 cm^{-1} region of the IR spectrum [8-10].

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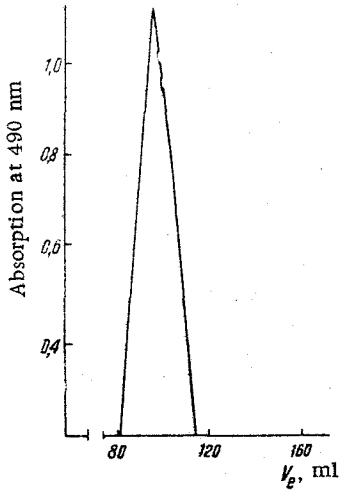


Fig. 1. Gel chromatography of glucofructan IVa from *A. sativum* on Sephadex G-75.

The IR spectrum of our glucofructan has absorption bands at 822, 860, and 940 cm^{-1}. The first and third bands are characteristic for glucofructans of the inulin type, while the band at 860 cm^{-1} is typical for levans. Consequently in the glucofructan of fraction IVa from *A. sativum* there are both types of bonds.

The ^{13}C NMR spectrum of fraction IVa* (Fig. 2) also shows the presence of 2 → 1 and 2 → 6 bonds. As we shall see from the facts given below, it contains peaks with chemical shifts corresponding to both 2 → 1- and 2 → 6-bound fructofuranose residues [20], (ppm):

	C-1	C-2	C-3	C-4	C-5	C-6
Residues of β-2 → 1-bound fructose units	62.2	104.7†	78.4	76.0‡	82.4	63.5
Residues of β-2 → 6-bound fructose units	62.0	105.15†	78.4	76.3‡	81.2	64.1

It also follows from the ^{13}C NMR spectrum that fraction IVa is not a mixture of glucofructans of the inulin and leval types, since in this case the spectrum would have no signals other than those mentioned above. In actual fact, there are also peaks at 104.8 ppm (C-2) and 76.7 ppm (C-4), belonging to the C-2 and C-4 atoms of end-to-end-linked units where 2 → 1- and 2 → 6-linked fructose residues are adjacent to one another.

Glucose is present at the reducing end of the polymer chain and is attached to the C-2 of fructose, as is shown by a peak with the chemical shift of C-1 of α-D-glucopyranose (93.3 ppm), which is characteristic for this type of attachment [12].

The quantitative ratio of 2 → 1 and 2 → 6 forms calculated from the integral intensities of the corresponding peaks is 1:1. The rate and completeness of the acid hydrolysis of the glucofructan confirms the furanose form of the D-fructose, and its negative rotation confirms the β configuration of the glycosidic bond.

When the peracetate of the glucofructan was oxidized with chromium trioxide in acetic acid [13, 14], no free fructose was detected in the oxidation products, which indicates a predominance of β-glycosidic bonds.

When fraction (IVa) was subjected to periodate oxidation, 0.90 mole of $NaIO_4$ was consumed and 0.088 mole of formic acid was formed per mole of anhydrohexose units. In the products of Smith degradation [15], glycerol and fructose were detected in a ratio of 13:1, respectively, by PC (system 2), and GLC (conditions A). The formation of glycerol is possible

*Interpretation in accordance with the literature [11].
†For end-to-end linked units 104.8 ppm.
‡For end-to-end linked units 76.7 ppm.

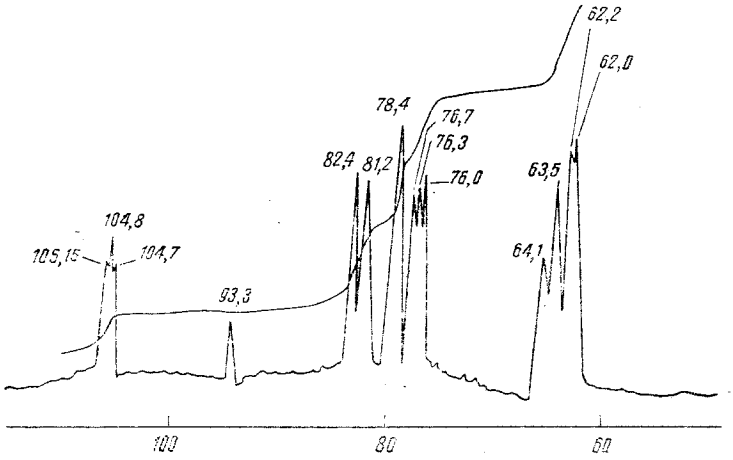


Fig. 2. ^{13}C NMR spectrum IVa from *A. sativum*.

in the case both of 2 → 1 and 2 → 6 links, while the presence of free fructose shows the existence of branching.

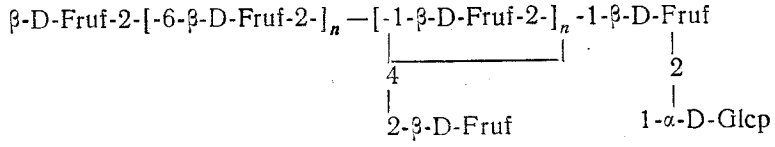
As is known [16, 17], mixtures of glucofructans with different structures but similar molecular weights can sometimes be separated by the fractionation of their peracetates. We separated the peracetate of our glucofructan by the successive addition of petroleum ether to an acetone solution, and obtained four fractions of the peracetate:

	A	*B*	*C*	*D*
Yield, %	16	66	9	9
$[\alpha]_D^{22}$, deg	—2,3	—2,25	—	—

Fractions A and B, making up the bulk of the peracetate, and also the initial glucofructan IVa, were subjected to Hakomori methylation [18]. Three identical permethylates with $[\alpha]_D^{22} \approx -4.2°$ were obtained. In the hydrolysates of all three permethylates, 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 3,6-di-O-methyl-D-fructose were detected in a ratio of 1:2:5:5:1, respectively, by TLC (conditions I and II) and GLC (conditions B and C). Since the tri-O-methyl derivatives of fructose give a single integral peak on determination by GLC, equal proportions of them in the mixture were assumed on the basis of the features of the ^{13}C NMR spectrum of the glucofructan and a visual evaluation of the intensities of the spots of the methylated sugars on TLC.

The formation of identical permethylates from the fractions of the peracetate and the initial glucofructan confirm the conclusions deduced from the ^{13}C NMR spectrum that we are dealing here with a single polysaccharide including two types of bonds and not with a mixture of two polysaccharides with different types of bonds. The presence of 3,4,6- and 1,3,4-tri-O-methyl-D-fructoses in the products of the hydrolysis of the permethylates also confirms the the indications of the IR and ^{13}C NMR spectra on the presence of equal numbers of 2 → 1 and 2 → 6 bonds in the glucofructan, and the detection of 3,6-di-O-methyl-D-fructose indicates branching at the C-4 hydroxyl in the inulin part of the molecule. In view of the ratio of methylated sugars and also the results of the Smith degradation, it can be stated that there is one branch to 12-13 fructose residues, i.e., per one molecule of glucofructan.

Thus it is possible to suggest the following general formula for the glucofructan of fraction IVA from *A. sativum*, where n is not more than 5:



EXPERIMENTAL

Solutions were evaporated in a rotary evaporator at a temperature of 40±5°C. IR spectra were recorded on a UR-20 instrument in tablets with KBr. Paper chromatography (PC) was carried out on FN-7 and –17 papers: by the descending method (1) with butan-1-ol–pyridine–water (6:4:3) and by the ascending method (2) with propan-1-ol–ethyl acetate–water (7:2:1). The following reagents were used to indicate the spots: 1) aniline hydrogen phthalate; 2) saturated solution of KIO_4–$KMnO_4$–benzidine.

The gas-liquid chromatography (GLC) of the samples was performed on a Tsvet-101 instrument with a flame-ionization detector.

Conditions: A) steel column (0.3 × 200 cm), Chromaton N-AW-DMCS (0.160 × 0.200 cm) impregnated with 5% of Silicone SE-30, temperature 180–220°C, nitrogen, 40 ml/min; B) steel column (0.3 × 200 cm). Chromaton N-AW-DMCS (0.160–0.200 cm), impregnated with 5% of Silicone XE-60, temperature 220–270°C, helium, 50 ml/min; C) column (0.3 × 200 cm), Chromaton N-AW-DMCS, impregnated with 5% of silicone XE-60, temperature 175–225°C, nitrogen, 120 ml/min.

Thin-layer chromatography (TLC) was performed on Silufol-254 in the following solvent systems: 1) benzene–acetone (2:1); 2) chloroform–methanol (9:1); 3) methyl ethyl ketone saturated with 1% aqueous ammonia (30:4). To indicate the spots we used o-toluidine salicylate, aniline phthalate, and the Bonner reagent [19].

^{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 with respect to protons. The samples were prepared as 3% solutions in D_2O, with methanol as internal standard, the chemical shift of which relative to TMS was taken as 50.15 ppm. The chemical shifts are given in the δ scale. The specific rotations were determined on a Zeiss polarimeter in a tube 0.5 dm long with a volume of 1 ml.

Isolation. The polysaccharides were obtained from the bulbs of *A. sativum* as described previously [1].

Fractionation. To 50 ml of a 5% solution of the polysaccharide were added successively 100, 150, 350, 650, and 750 ml of ethanol, giving fractions I, II, III, IVa, and IV.

Gel Chromatography. Samples (20 mg in 2 ml of distilled water in each case) of the glucofructan from *A. sativum*, raffinose, inulin, and a dextran with mol. wt. 10,000 were deposited on a column of Sephadex G-75 (56 × 2 cm). Elution was carried out with the same solvent. The column of Sephadex G-75 was calibrated from the breakthroughs of inulin (mol. wt. 5600, V_e = 84.1 ml), of raffinose, mol. wt. 504, V_e = 127 ml), and of dextran, mol. wt. 10,000, V_e = 75.5 ml). Eluates with a volume of 3 ml each were collected over 15 min and were analyzed by the phenol–sulfuric acid method.

The molecular weight of fraction IVa from *A. sativum* (V_e = 97.2 ml) was determined from a graph of the dependence of log M_n on V_e as 2300.

Hydrolysis. A mixture of 50 mg of fraction IVa of the polysaccharide in 5 ml of 0.1 M H_2SO_4 was heated in the boiling water bath for an hour. Then the hydrolysate was neutralized with calcium carbonate and was treated with KU-8 cation-exchange and AN-31 anion-exchange resins, concentrated in vacuum, and chromatographed on FN-17 paper (system 1), and it was subjected to GLC analysis in the form of the TMS ethers (conditions A).

Periodate oxidation and Smith degradation were carried out as previously [1]. The consumption of periodate amounted to 0.088 mole per mole of anhydrohexose units. In the products of Smith degradation, glycerol and fructose were detected in a ratio of 13:1.

Acetylation. Fraction IVa from *A. sativum* (1.0 g) was dissolved in pyridine (5 ml), and, with cooling, 4 ml of acetic anhydride in 1-ml portions. After the addition of the acetic anhydride, the mixture was stirred for three days. Then it was poured into iced water. The resulting precipitate was washed with water and dissolved in chloroform, and the solution was evaporated to a syrup (1.1 g). After drying in vacuum, the completeness of acetylation was checked by the IR-spectroscopic method.

Chromium Trioxide Oxidation. The peracetate of fraction IVa, 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° for 4 h [14]. An ill-defined spot of glucose was detected by the PC method in System 1.

Fractionation of the Peracetate of Glucofructan IVa from *A. sativum*. A solution of 0.9 g of peracetate in 1.05 ml of acetone and 1.65 ml of chloroform was treated successively with 40, 25, 40, and 60 ml of petroleum ether. This gave four fractions — A, B, C, D.

Methylation by Kakomori's method was applied to the initial fraction IVa (0.6 g) and a part of the peracetate of fraction A (0.14 g) and B (0.6 g). The completeness of methylation was checked by IR spectroscopy. After two methylations, the yields of permethylates were 0.45, 0.10, and 0.4 g, respectively.

Analysis of the Methylation Products. The permethylates were hydrolyzed with 0.5% H_2SO_4 for 8 h, and the hydrolysates were neutralized with calcium carbonate. The concentrated syrups were subjected to TLC in systems 1 and 3 and were separated into individual components which were compared with authentic samples. The homogeneity of the methyl ethers obtained was checked by TLC in system 2. Under the action of the Bonner reagent [19], 1,3,4-tri-O-methylfructose is not oxidized, unlike 3,4,6-tri-O-methylfructose and 3,6-di-O-methylfructose. The mixture of methylated sugars was reduced and was determined by GLC in the form of the acetates and trifuroacetates of the polyols so obtained.

Tetra-O-Me-Glc, tetra-O-Me-Fruf, tri-O-Me-Fruf, and di-O-Me-Fruf were detected in a ratio of 1:2:10:1.

CONCLUSION

By fractionating the combined glucofructans from the bulbs of *Allium sativum* L. a homogeneous glucofructan with a molecular weight of 2300 has been isolated. It has been shown by spectral and chemical methods that it consists of a new type of compounds of this class containing inulin (2 → 1) and levan (2 → 6) glycosidic bonds.

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