PARTIAL METHYLATION OF METHYL α-D-GLUCOPYRANOSIDE.

II. PREPARATIVE LIQUID AND GAS-LIQUID CHROMATOGRAPHY OF THE ACETATES OF THE MONO- AND DI-O-METHYL ETHERS OF METHYL α-D-GLUCOPYRANOSIDE

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

A method has been described [1] for obtaining the acetates of the tri-O-methyl ethers of methyl α-D-glucopyranoside with the aid of liquid chromatography. We are continuing the study of the possibilities of liquid and gas-liquid chromatography for the preparative production of the mono- and di-O-methyl ethers of methyl α-D-glucopyranoside.

The acetates of the monomethyl ethers are partially separated in a thin layer of silica gel, which leads to the formation of two compounds with R_f 0.34 and 0.45. To separate the acetates of the monomethyl ethers into two fractions we used liquid chromatography on silica gel. The separation gave two fractions. The first contained the acetate of the 2-O-methyl ether, and the second a mixture of acetates of the 3-, 4-, and 6-O-methyl ethers. The latter were separated by preparative GLC.

In the separation of the acetates of the di-O-methyl ethers in a thin layer, a partial separation of the mixture was observed with the formation of five spots. The preparative liquid chromatography of a mixture of the acetates of the di-O-methyl ethers yielded in the individual states the acetates of the 3,4-, 2,3-, and 2,6-di-O-methyl ethers. A mixture of the acetates of the 2,4- and 2,6-di-O-methyl ethers was separated by preparative GLC. The large differences in the retention times between the successive di-O-methyl ethers permitted the load on the column (200 × 1.4 cm) to be raised to 300 mg of the mixture. At the same time, it does not appear possible to separate the mixture of the acetates of the 3,6- and 4,6-di-O-methyl ethers on a preparative GLC column because of the insignificant differences in their retention times. These ethers were obtained in the following way. From a mixture of the two compounds, the 4,6-di-O-methyl ether was obtained by crystallization from ether. This mixture was deacetylated, oxidized with periodiate, re-acetylated, and separated by preparative GLC. As a result, the 3,6-di-O-methyl ether was obtained in the individual state. Thus, the separation of all the di-O-methyl ethers has been achieved. Analytical GLC demonstrated the chromatographic purities of the compounds obtained.

EXPERIMENTAL

The identification of the acetates of the methyl ethers of methyl α-D-glucopyranoside was carried out by comparison with authentic samples, and also by using the results of chromato-mass spectrometry [2]. The general experimental conditions have been given in our preceding paper [1].

Preparative GLC. For preparative GLC we used a PAKhV-07 instrument (Institute of Petrochemical Synthesis), fitted with a katharometer and a glass column (200 × 1.4 cm). As the liquid phase we used 5% and 10% of NPGS on Chromaton NAW HMDS (0.20-0.25 mm, Chemapol). The rate of flow of helium was 400 ml/min, the temperature of the evaporator was 300°C and that of the collector was 120°C. Uncooled glass ampuls (80 × 5 mm) were used as receivers.

Separation of the Acetates of the Monomethyl Ethers of Methyl-D-glucopyranoside. A mixture (11.2 g) of the acetates of the monomethyl ethers was deposited on a column (45 ×

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3 cm) of silica gel and was eluted with the following gradient of solvents, using 0.6 liter of each: 10%, 15%, 20%, and 50% of ethyl acetate in petroleum ether (mp 70-100°C). Fractions with a volume of 50 ml each were collected. The rate of elution was 7 ml/min. The results of the separation were:

Methyl ether	Fraction No.	Yield, g	R_f
3+4+6	10—23	5.5	0.45
3+4+6+2	24—35	1.4	
2	36—46	3.9	0.34

For the acetate of the 2-O-methyl ether, mp 122-123°C, $[\alpha]_D^{20}$ +152.7° (c 0.9). According to the literature [3]: mp 120°C, $[\alpha]_D^{20}$ + 145°.

The acetate of the 2-O-methyl ether (0.2 g) was dissolved in absolute methanol (2 ml), and 0.05 ml of a 0.1 N solution of sodium methanolate was added. Deacetylation was complete after 40 min at room temperature (checked by TLC). The solution was deionized with KU-2 cation-exchange resin in the H^+ form, filtered from the resin, and evaporated. The 2-O-methyl ether was obtained after recrystallization from ethyl acetate with a yield of 93 mg, mp 147-148°C, $[\alpha]_D^{20}$ +155.0° (c 1.1). According to the literature: 147-148°, $[\alpha]_D^{20}$ +155.0°.

A mixture of the acetates of the 3-, 4-, and 6-O-methyl ethers was separated by preparative GLC on a column (200 × 1.4 mm) containing 5% of NPGS at 180°C. The load on the column was 100 mg of mixture in the form of a 50% solution in chloroform. The yields and properties of the acetates of the monomethyl ethers were as follows:

Methyl ether	Amount in the mixture, %	Yield, mg	$[\alpha]_D^{20}$
3	12.3	6	+112.1° (c 1.8)
4	28.2	13	+92.1° (c 1.7)
6	59.5	29	+135.9° (c 2.0)

Methyl 2,4,6-tri-O-acetyl-3-O-methyl-α-D-glucopyranoside was recrystallized from a mixture of dimethyl ether and petroleum ether (bp 70-100°C); mp 67-68°C.

Separation of the Acetates of the Di-O-methyl Ether of Methyl α-D-Glucopyranoside. A mixture (10.5 g) of the acetates of the di-O-methyl ethers was deposited on a column (45 × 3 cm) of silica gel and was eluted with the following gradient of solvents, using 0.8 liter of each: 5%, 7.5%, 10%, 15%, and 20% ethyl acetate and petroleum ether (bp 70-100°C). Fractions with a volume of 50 ml each were collected, the rate of elution being 70 ml/min. The results of the separation were:

Methyl ether	Fraction No.	Yield, g	$[\alpha]_D^{20}$	R_f
3,4	25—31	0.48	131.6° (c 2.1)	0.52
3,4+3,6+4,6	32—43	2.75		
3,6+4,6	44—56	2.70		0.45
3,6+4,6+2,3	57—65	0.95		
2,3	66—73	0.55	104.2° (c 1.8)	0.35
2,3+2,6	74—76	0.26		
2,6	77—82	0.95	156.6° (c 2.2)	0.32
2,4+2,6	83—89	1.32		

A mixture of the acetates of the 2,4- and 2,6-di-O-methyl ethers (300 mg) in the form of a 50% solution in chloroform was separated by preparative GLC on a column (200 × 1.4 cm) containing 10% of NPGS at 170°C. The acetate of the 2,4-di-O-methyl ether was obtained with a yield of 62 mg, $[\alpha]_D^{20}$ +123.0° (c 2.8). The yield of the acetate of the 2,6-di-O-methyl ether was 78 mg, $[\alpha]_D^{20}$ +153.1° (c 3.0).

A mixture (2.7 g) of the acetates of the 3,6- and 4,6-di-O-methyl ethers of methyl α-D-glucopyranoside was crystallized from ether. The acetate of the 4,6-di-O-methyl ether was obtained in the crystalline state with a yield of 2.1 g, mp 53.5-54.0°C, $[\alpha]_D^{20}$ +118.7° (c 2.8). The crystals were separated off, and the mother solution was evaporated. The residue (0.6 g) was dissolved in 10 ml of absolute methanol, and the solution was treated with 0.1 ml of a 0.2 N solution of sodium methanolate. The mixture was kept at room temperature for 40 min and it was then deionized with KU-2 cation-exchange resin (H^+), filtered from the resin, and evaporated. The resulting syrup (0.39 g) was dissolved in 10 ml of water, 0.1 ml of 0.07 N periodic acid was added, and the mixture was left in the dark for 12 h. Then it

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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