nol was used as internal standard (50.15 ppm). The volume of the memory was 8/4 K, the length of pulse 10 usec (85°), the pulse repetition frequency 0.7 sec, the width of the spectrum 3750 Hz, and the number of accumulations 100-150 thousand.

#### SUMMARY

The <sup>13</sup>C NMR spectra of the glucomannans from E. lactiflorus and E. luteus have been analyzed in comparison with the spectra of low-molecular-weight model compounds. It has been shown that the linear polymeric chain consists of  $1\rightarrow 4-\beta$ -linked glycoside residues in the pyranose form, which confirms the results obtained previously from chemical investigations of these polysaccharides.

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PARTIAL METHYLATION OF METHYL  $\alpha$ -D-GLUCOPYRANOSIDE.

I. LIQUID CHROMATOGRAPHY OF THE ACETATES OF THE TRI-

O-METHYL ETHERS OF METHYL  $\alpha$ -D-GLUCOPYRANOSIDE

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A method is described for obtaining the acetates of the tri-O-methyl ethers of methyl  $\alpha$ -D-glucopyranoside via the partial methylation of methyl  $\alpha$ -D-glucopyranoside and the subsequent preparative liquid chromatography of the acetates of the tri-O-methyl ethers.

At the present time, methods have been described for obtaining methyl ethers of monosaccharides which are based on the partial methylation of methyl glycosides followed by chromatography of the mixtures obtained [1-6]. For this purpose the preparative GLC of methyl ethers of methyl  $\alpha$ -D-mannopyranoside [1], of methyl  $\beta$ -D-xylopyranoside [2], of methyl  $\alpha$ -Dgalactopyranoside [3], and of methyl  $\alpha$ -L-rhamnopyranoside [4] have been used. Liquid chromatography on silica gel has been employed successfully for the separation of methyl ethers of methyl  $\alpha$ -L-rhamnopyranoside [5]. An advantage of liquid chromatography on silica gel in comparison with GLC consists in the possibility of using larger amounts of substances. However, the slight differences in the chromatographic mobility of the methyl ethers do not permit the wide use of this method. With the aid of liquid chromatography on silica gel we have succeeded in achieving the separation of the acetates of the tri-O-methyl ethers of methyl  $\alpha$ -Dglucopyranoside and of obtaining all the tri-O-methyl ethers in the individual state with good yields.

The partial methylation with dimethyl sulfate in alkali of 50 g of methyl  $\alpha$ -D-glucopyrannoside yielded 31 g of a mixture of methyl ethers soluble in the organic phase and 22 g soluble in the aqueous phase. It was shown by TLC that the organic phase (fraction A) contained tetra-, tri-, di-, and mono-O-methyl ethers, and the aqueous phase (fraction B) di- and mono-

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O-methyl ethers, and also the initial glycoside. By liquid chromatography on silica gel, the fractions obtained were separated into components with the same degree of substitution with a yield of 95%, and for the subsequent treatment they were combined according to their degree of substitution.

GLC does not ensure the complete separation of the acetates of the methyl ethers, and under the conditions of GLC no separation of the methyl ethers into functions with the same degree of substitution is observed. Consequently, we investigated the possibility of using liquid chromatography on silica gel for the separation of methyl ethers with the same degree of substitution in the form of their acetates. Below we give the  $R_f$  and  $R_t$ \* values of the acetates of the methyl ethers of methyl  $\alpha$ -D-glucopyranoside:

Methyl ether	$R_{f}$	$R_t^*$
2,3,4,6	0,33	0,05
2,3,4	0,36	0,15
2,3,6	0.31	0,16
2,4,6	0,28	0,31
3,4,6	0.50	Q.12
2,3	0,35	0,43
2.4	0,29	1,00
2,6	0, 32	0,50
3.4	0,52	0,42
36	0,44	0,43
4,6	0,46	0,50
2	0,34	1,26
3	0,45	1,14
4	0,44	1.61
6	0,46	0,83
a-D-Glc	0,47	2,07

It can be seen from these figures that because of the close  $R_f$  values it is impossible to separate all the components of the mixture on one column, but the acetates of the methyl ethers with the same degree of substitution can be separated because of the small numbers of components.

By using liquid chromatography on silica gel, we have shown the possibility of satisfactorily separating the tri-O-methyl ethers in the form of acetates when the column ( $45 \times 3$  cm) is loaded with up to 10 g of the mixture. The total yield and the yields of the individual methyl ethers amounted to 97.6% and 57.9%, respectively. Analytical GLC demonstrated the chromatographic purity of all the methyl ethers obtained.

# EXPERIMENTAL

The methyl ethers of methyl  $\alpha$ -D-glucopyranoside were identified by mass spectrography [6]. Specific rotations were measured on a Perkin-Elmer M 141 instrument, using chloroform as the solvent. Melting points were measured on a Boetius instrument. TLC was performed on silica gel L 5/40  $\mu$  (Chemapol). The following solvent systems were used: for the methyl ethers of methyl  $\alpha$ -D-glucopyranoside: chloroform-methanol (9:1); for the acetates of the methyl ethers of methyl  $\alpha$ -D-glucopyranoside: petroleum ether (bp 70-100°)-ethyl acetate (1:1). The spots were revealed with a 30% solution of sulfuric acid in methanol. For column chromatography we used silica gel L 100/160  $\mu$  (Chemapol), previously fractionated. The rate of elution was 7 ml/min, and 50-ml fractions were collected. GLC was performed on a Tsvet-106 instrument fitted with a flame-ionization detector and with double columns (200 × 0.3 cm). As the liquid phase we used 3% of NPGS on Chromaton NAW-HMDS (0.125-0.160 mm). The rate of flow of argon was 30 ml/min, and the thermostat temperature was 165°C. The samples were acetylated with acetic anhydride in pyridine.

Partial Methylation. Methyl  $\alpha$ -D-glucopyranoside (50 g) was dissolved in the minimum volume of water and, with stirring by means of a magnetic stirrer in a current of argon, 100 ml of dimethyl sulfate and 170 ml of 170 ml of 30% aqueous caustic soda were added to the solution dropwise over an hour. The reaction mixture was kept in the water bath for 30 min, and it was then cooled and neutralized with concentrated sulfuric acid. The volume of solution was brought to 500 ml and it was extracted with chloroform (500 ml  $\times$  2), and the organic phase and the chloroform extracts were evaporated. This gave fraction A (yield 31 g). The aqueous phase was deionized with AV-17 (OH-) and KU-2 (H<sup>+</sup>) resins and evaporated. This gave fraction B (yield 22 g).

\*Retention time relative to the acetate of methyl 2,4-di-O-methyl- $\alpha$ -D-glucopyranoside (21.8 min).

Separation of Fraction A. Fraction A (21 g) was deposited on a column ( $45 \times 3$  cm) of silica gel and was eluted with the following gradient of solvents, using 0.5 liter of each: petroleum ether (bp 70-100°C), petroleum ether-chloroform (1:1); chloroform; and 2%, 5%, 7%, and 10% of methanol in chloroform. Fractions with a volume of 50 ml each were collected. The course of elution was monitored with the aid of TLC. We give the yields of the methyl ethers:

Methyl ethers	Fraction No.	Yield, g	
2, 3, 4, 6,	15-21	2.7	
Tri	2337	8,9	
Di	3965	7.6	
Mono	7175	0.6	

Methyl 2,3,4,6-tetra-0-methyl- $\alpha$ -D-glucopyranoside consisted of a colorless group,  $\alpha[_{D}^{2\circ}]$  + 141.7° (c 5.4).

Separation of Fraction B. Fraction B (20 g) was deposited on a column ( $45 \times 3$  cm) of silica gel and was eluted with the following gradient of solvents, using 0.5 liter of each: chloroform, and 2%, 5%, 10%, and 20% of methanol in chloroform. The results of the separation are given below:

Methyl ethers	Fraction No.	Yield, g
Di	11-17	4,0
Di + mono	18-19	1,5
Mono	20 - 25	11,5
a-D-Gle	28-30	2.0

Separation of the Acetates of the Tri-O-methyl Ethers. A mixture of the acetates of the tri-O-methyl ethers of methyl  $\alpha$ -D-glucopyranoside (9.7 g) was deposited on a column (45 × 3 cm) containing silica gel and was eluted with the following gradient of solvents, using one liter of each: petroleum ether, and 2%, 5%, 10%, and 20% of ethyl acetate in petroleum ether. The yields and properties of the acetates of the tri-O-methyl ethers of methyl  $\alpha$ -D-glucopyranoside are as follows:

Methyl ether	Fraction No.	Yield, g	$[\alpha]_D^{20}$	mp
3 4,6	40-47	0,6	$+134.8^{\circ}$ (c 1,1)	syrup
2, 3, 4+3, 4, 6	48-52	0.4	+ 121 19 (0 1 4)	eVrup
2, 3, 4 2, 3, 4+2, 3, 6	53 - 58 59-62	0.5	+151,1 (01,4)	syrup
2, 3, 6	63-66	14	$+117,0^{\circ}$ (c 1,0)	57,5-58,00
$2, 3, 6+2, 4, 6 \\2, 4, 6$	$67 - 72 \\ 73 - 86$	$\frac{1}{2.2}$	+148.2° (c 1,5)	syrup
	-			

# CONCLUSION

A method has been described for obtaining the acetates of the tri-O-methyl ethers of methyl  $\alpha$ -D-glucopyranoside which is based on the partial methylation of methyl  $\alpha$ -D-glucopyranoside with the subsequent preparative liquid chromatography of the acetates of the tri-O-methyl ethers.

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