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POLYSACCHARIDES OF *Eremurus*.

XV. STRUCTURE OF THE GLUCOMANNAN OF *Eremurus lactiflorus*.

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Ten oligosaccharides have been isolated from the products of the partial hydrolysis of a native acetylated glucomannan obtained from *Eremurus lactiflorus* O. Fedtsch. Their structures have been studied with the aid of acid hydrolysis before and after reduction with $NaBH_4$, by the GLC method, and also by chromatography with markers. The compositions and sequence of the monomers in tetra- and heptaoligosaccharides have been determined by the ^{13}C NMR method. The glucomannan of *E. lactiflorus* differs from the *Eremurus* glucomannans studied previously by the ratio of monosaccharides, the presence of O—Ac groups, the degree of polymerization, and the presence of a cellobiose unit (Glcp—Glcp) in the polymer chain. The repeating unit consists of 14 hexose residues.

To establish the sequence of monosaccharides in the chain of a glucomannan isolated from the tuberous roots of *Eremurus lactiflorus* O. Fedtsch. [1, 2], it has been subjected to partial acid hydrolysis. Several oligosaccharides, in addition to glucose and mannose, were detected in the hydrolysis products by paper chromatography (PC).

The total oligosaccharides were separated into fractions with the aid of gel filtration on Sephadex G-15, and the fractions obtained were then subjected to preparative PC. In this way oligosaccharides designated A—I were isolated (Table 1).

The structure of oligosaccharides A—E were studied with the aid of complete acid hydrolysis before and after reduction with $NaBH_4$, and also by paper chromatography with markers. The compositions and the sequence of monomers in oligosaccharides F—I were also studied by the ^{13}C NMR method.*

Figures 1 and 2 show the ^{13}C NMR spectra of oligosaccharides F, G, H, and I. In the assignment of the signals in the spectra we started from the following assumptions:

1. The spectra of mannose and glucose residues in the center of an oligosaccharide coincide with the spectra of the corresponding residues in the polysaccharide chain [3];

2. the spectra of β-D-mannopyranose and β-D-glucopyranose residues at the nonreducing ends of oligosaccharides differ from the spectra of the corresponding residues in the polymer chain in the same way as the spectra of methyl β-mannopyranoside and methyl β-glucopyranoside differ from the spectra of 4-O-methyl-β-D-mannopyranose and 4-O-methyl-β-D-glucopyranose [4, 5];

*The ^{13}C NMR spectra were taken by A. S. Shashov of the N. D. Zelinskii Institute of Organic Chemistry, Academy of Sciences of the USSR.

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TABLE 1. Oligosaccharides Formed in the Partial Hydrolysis of the Glucomannan

Oligosaccharide	R_{Man}	Composition and ratio of the monomers*	Terminal residue	DP	Type and configuration of the bond	Structure
A	0,83	G, M (1:1)	M	2	β-(1→4)	G →M
B	0,67	G	G	2	β-(1→4)	G →G
C	0,53	M	M	2	β-(1→4)	M → M
D	0,46	G, M (1:1)	G	2	β-(1→4)	M→G
E	0,36	G, M (1:2)	M	3	β-(1→4)	G →M→ M
F	0,29	G, M (1:3)	M	4	β-(1→4)	G →M→M→ M
G	0,20	G, M (2:3)	M	5	β-(1→4)	M →G→G→M →M
H	0,16	G, M (1:4)	M	5	β-(1→4)	M →G→M→M →M
I	0,07	G, M (3:4)	M	7	β-(1→4)	G →M→G→G→M →M→ M

*G—Glcp, M—Manp.

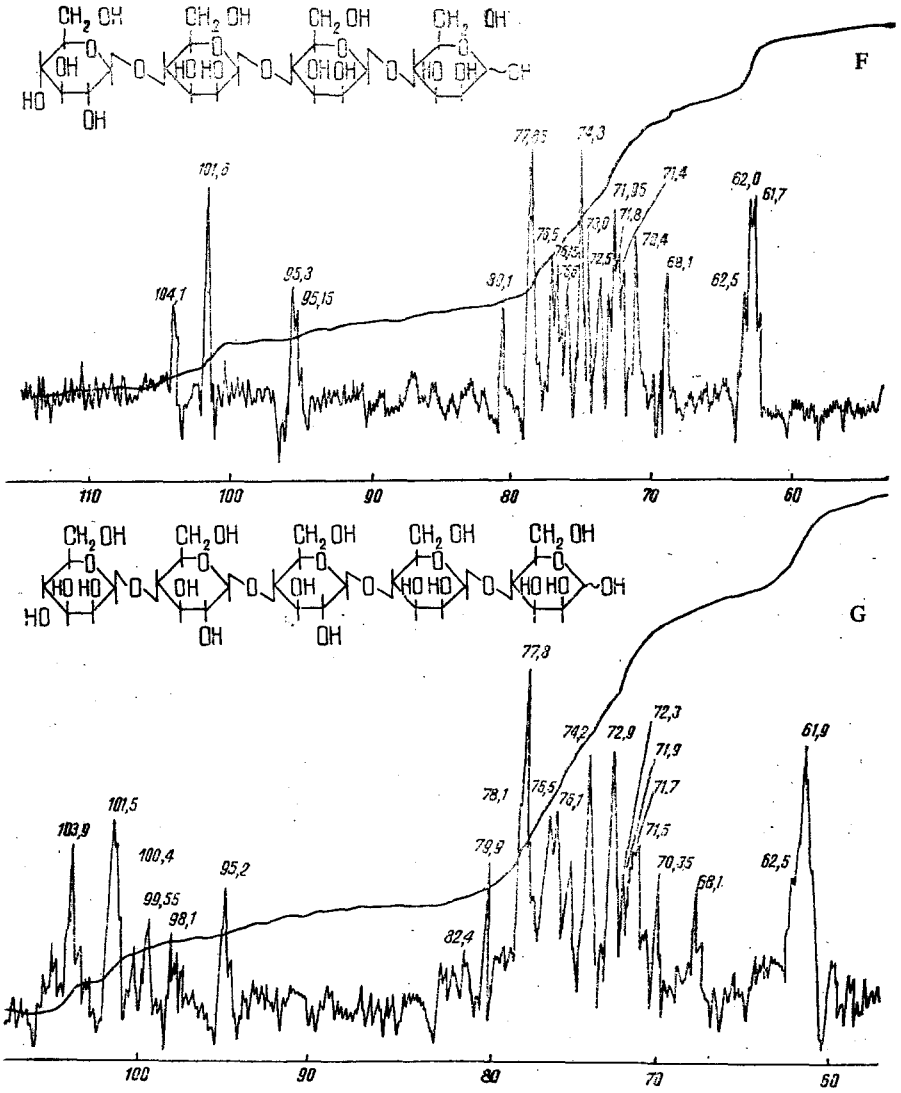

Fig. 1. ^{13}C NMR spectra of oligosaccharides F and G.

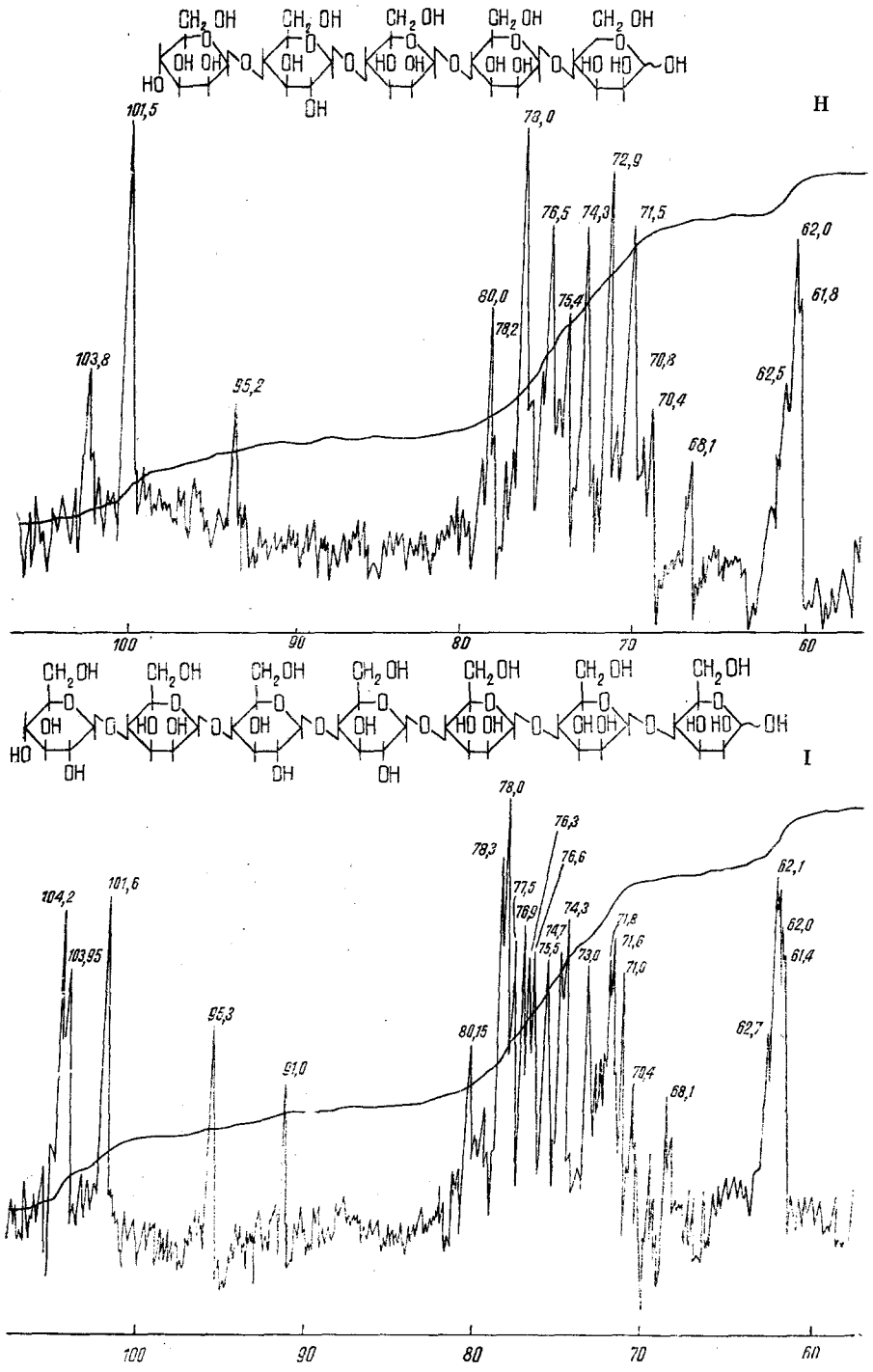

Fig. 2. ^{13}C NMR spectra of oligosaccharides H and I.

3. The spectra of mannose and glucose residues at reducing ends of oligosaccharides differ from the spectra of the corresponding residues in the polymer chain in the same way as the spectra of 4-O-methyl-α- and -β-D-mannopyranoses and of 4-O-methyl-α- and -β-D-glucopyranoses differ from the spectra of the corresponding pyranosides.

In the region of the resonance of the anomeric protons of the carbon spectra of the oligosaccharide that was considered, there are signals characteristic only for terminal α-, and β-mannopyranose residues (95.1–95.3 ppm) and no signals of terminal β- and α,β-glucopyranose residues (97 ppm and 93 ppm, respectively) [6]. On the other hand, all the spectra

contain the signals of the C_4 atom with an unsubstituted hydroxyl group of β-D-mannopyranose and β-D-glucopyranose residues (68.0–68.1 ppm) and (70.4–70.5 ppm), respectively.

The compositions of the oligosaccharides were analyzed in the following way: signals in the 103.8–104.1 ppm region relate only to the C_1 atoms of glucose residues and those at 101.45–101.6 and 95.1–95.3 only to the C_1 atoms of mannose residues. The ratio of the integral intensities of the peaks in the first region to the total integral intensity of the peaks in the other two regions gives the ratio of glucose and mannose units in the oligosaccharide fractions concerned.

In addition, the ratio of the integral intensity in the region of resonance of the C_1 atoms of α,β-mannopyranose residues at reducing ends (95.1–95.3 ppm) to the total intensity of the peaks from the C_1 atoms of β-mannopyranoside residues (101.5–101.6 ppm) and of β-glucopyranose residues (103.8–104.2 ppm) permits the number-average molecular masses of the oligomer fractions to be determined. The results of an analysis of the spectra are given below:

103.8–104.2	C 1 Glcp β	At the nonreducing end and in the middle of the chain
101.45–101.6	C 1 Manp α, β	
95.1–95.3	C 1 Manp	At the reducing end
75.95–80.15	C 4 Glcp β	In the middle of the chain
78.1–78.3	C 5 Manp β	At the nonreducing end
77.4–78.0	C 4 5 Manp β	At the reducing end and in the middle of the chain
	C 4 Manp α	At the reducing end
76.4–76.9	C 3 Glcp β	At the nonreducing end and in the middle of the chain
76.15–76.3	C 5 Glcp β	At the nonreducing end
75.4–75.5	C 5 Glcp β	In the middle of the chain
74.3–74.7	C 2 Glcp β	At the nonreducing end and in the middle of the chain
72.9–73.1	C 3 Manp β	
72.5–72.6	C 5 Manp α	At the reducing end
71.4–72.0	C 2 Manp β and Manp α	At the reducing end
71.0–71.1	C 3 Manp α	At the reducing end
70.4–70.5	C 4 Glcp β	At the nonreducing end
68.0–68.1	C 4 Manp β	
62.4–62.5	C 6 Manp β	
61.7–62.1	C 6 Manp α, β	At the reducing end and Manp-β in the middle of the chain
	C 6 Glcp β	At the nonreducing end and in the middle of the chain

The glucomannans of *E. lactiflorus* O. Fedtsch. differ from the glucomannans considered previously [7, 8] by the ratio of monosaccharides, by the presence of O–Ac groups, by the degree of polymerization, and by the presence of a cellobiose unit (Glcp–Glcp) in the polymer chain. The repeating unit consists of 14 hexose residues: $-[M \rightarrow M \rightarrow M \rightarrow G \rightarrow M \rightarrow G \rightarrow G \rightarrow M \rightarrow M \rightarrow M \rightarrow G \rightarrow M \rightarrow M \rightarrow M]_n-$.

EXPERIMENTAL

Solutions were evaporated in a rotary evaporator at 40 ± 5°C. Paper chromatography was performed on Filtrak 1, 11, 7, and 16 papers by the descending method using the solvent system butan-1-ol–pyridine–water (6:4:3). The spots were revealed with aniline hydrogen phthalate (10–15 min at 105–110°C) [9]. The GLC of the samples was carried out on a Tsvet-101 instrument under the conditions described in our previous paper [1].

The ^{13}C NMR spectra were taken on a WP-60 instrument with a working frequency of 15.08 MHz under the conditions described for a glucomannan [3].

Partial Hydrolysis. The glucomannan (7 g) was hydrolyzed in 100 ml of 0.5 N H_2SO_4 (90°C, 2 h), and the reaction mixture was neutralized with a slurry of $BaCO_3$. The precipitate was filtered off and the filtrate was evaporated. Paper chromatography showed the presence in the mixture of mannose, glucose, and nine oligosaccharides. The mobilities of the oligosaccharides with respect to mannose are given in Table 1.

Gel Filtration. The mixture of oligosaccharides (0.5 g) was separated on a column (60 × 3.0 cm) of Sephadex G-15 with elution by water, 3-ml fractions being collected. Separation was monitored by the phenol–sulfuric acid method [10]. The fractions corresponding to peaks on the elution curve were combined and evaporated, and the residues were then

analyzed by the PC method. Fractions containing oligosaccharides were separated by preparative PC. A total of 5 g of the mixture of oligosaccharides was separated.

The oligosaccharides were hydrolyzed by heating 10 mg of the sample in 1 ml of 1 N H_2SO_4 on the boiling water bath for 2 h, and the hydrolysate was then neutralized with $BaCO_3$, filtered, treated with KU-2 (in the H^+ form), evaporated, and chromatographed.

An oligosaccharide (10 mg), dissolved in 5 ml of water, was reduced with 20 mg sodium tetrahydroborate for 12 h, the excess of $NaBH_4$ was decomposed with Amberlite IR-120 (H^+), the boric acid was eliminated in the form of methyl borate by evaporation with methanol, and the residue was hydrolyzed under similar conditions.

The degrees of polymerization of the oligosaccharides A–E were determined by GLC from the ratio of reducing sugars before and after the reduction of the oligosaccharides with sodium tetrahydroborate.

SUMMARY

Partial hydrolysis of the glucomannan has yielded nine oligosaccharides, and their monomeric compositions and degrees of polymerization have been determined. The structures of the oligosaccharides have been studied by ^{13}C NMR spectroscopy, and on the basis of the results the structure of the repeating unit of the glucomannan from *Eremurus lactiflorus*, consisting of 14 hexose residues, has been given.

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