POLYSACCHARIDES OF *Eremurus*.

XI. PRODUCTS OF THE OXIDATION AND METHYLATION OF A GLUCOMANNAN FROM *Eremurus lactiflorus*

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It has been established by oxidation with chromium trioxide and sodium periodate and by methylation that the monosaccharide residues in the glucomannan of *Eremurus lactiflorus* O. Fed. are linked by β-1 → 4 bonds, and there is a mannose residue at the nonreducing end. The glucomannan macromolecule has a linear structure.

The isolation of polysaccharides from the tuberous roots of *Eremurus lactiflorus* O. Fed. and the nature of fraction 1 (A-2) have been reported previously. Continuing investigations in this direction we have characterized another fraction and have studied the structure of A-2.

By fractionation of a 1% aqueous solution of the neutral polysaccharides free from uronic acids by precipitation with ethanol, four fractions with the properties given in Table 1 were obtained.

As can be seen from the figures given, with a fall in the molecular weight of the glucomannan fractions the relative viscosity decreased and the specific rotation and the mannose content increased. According to their IR spectra, all the glucomannan fractions contained O-acyl groups.

The structure of the glucomannan of fraction 1 (A-2) [1] was studied by periodate and chromic oxidation and by methylation. The periodate oxidation of A-2 at room temperature (20-22°C) took place slowly (Fig. 2) and was complete only after 192 h. The consumption of sodium periodate then amounted to 0.94 mole per mole of anhydrohexose unit, and the amount of acid liberated was 0.065 mole.

The Smith degradation [2] of the oxidation product formed mainly erythritol and traces of glycerol, but no monosaccharides were detected. Consequently, in the main chain of the glucomannan 1 → 4 bonds predominate and there is no branching.

*Deceased.

TABLE 1

Fraction	Total volume of alcohol added	Yield, %	$[\alpha]_D^{20}$, °C (in water)	η_{rel} c (1; H_2O)	Ratio of glucose and mannose (GLC)	Molecular weight
1	75	58	—21.7 (*c* 0.73)	4,8	1:2,8	79000
2	125	26	—37 (*c* 1,0)	3,0	1:4,2	43000
3	225	10	—44 (1.0)	1.5	1:8	35000
4	500	4	—49 (*c* 0.4)	0,53	1:5,5	

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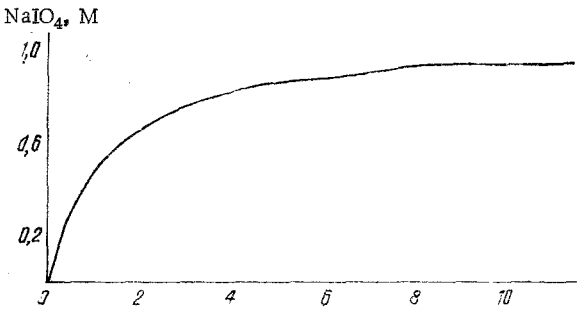

Fig. 1. Graph of the periodate oxidation of glucomannan A-2.

To determine the configuration of the glycosidic bonds we used the method of oxidizing the acetylated polysaccharides with chromium trioxide [3] under which conditions β-glycosidic bonds are oxidized. We detected no hexoses by PC in the oxidation products. It follows from this that in the glucomannan the glucose and mannose residues are linked by β-glycosidic bond

The glucomannan was methylated twice by Haworth's method [4, 5], and then by Kuhn's method [6], and methylation was brought to completion by Purdie's method [7]. The permethylate was white amorphous powder soluble in tetrahydrofuran, acetone, and chloroform, and insoluble in water, $[\alpha]_D^{20}$ −20° (c 1.0; $CHCl_3$). The high specific rotation of the permethylate also indicated the β-configuration of the glycosidic bonds. Its IR spectrum lacked the absorption band of a hydroxy group. The content of methoxy groups amounted to 40.88%. The methylated glucomannan was subjected to formolysis and hydrolysis, and in the resulting products 2,3,6-tri-O-methyl-D-mannose, 2,3,6-tri-O-methyl-D-glucose, and 2,3,4,6-tetra-O-methyl-D-mannose were found by TLC. The ratio of the 2,3,6-tri-O-methyl-glucose and -mannose was 1:2.8 (according to the GLC of the corresponding polyol acetates) which agrees with the ratio of the monosaccharides in a hydrolysate of the glucomannan A-2. The results of a study of th methylation and sodium periodate oxidation products permit the conclusion that the hexose residues in the glucomannan are connected by β-1 → 4 bonds and there is a mannose residue at the nonreducing end of the glucomannan.

EXPERIMENTAL

Solutions were evaporated in a rotary evaporator at 40 ± 5°C. Descending chromatography was carried out on types FN-7 and 11 papers, and thin-layer chromatography (TLC) on Silufol plates of type UV-245. The following solvent systems were used (ratios by volume): 1) butan-1-ol—pyridine—water (6:4:3); 2) ethyl acetate—propanol—water (1:7:2); 3) benzene—acetone—water (5:5:1); 4) chloroform—methanol (4:1); 5) methyl ethyl ketone—1% ammonia (30:4) Sugars and their derivatives were revealed with 1) a solution of aniline hydrogen phthalate; 2) a mixture of periodate, $KMnO_4$, and benzidine; and 3) concentrated sulfuric acid at 105°C. The IR spectrum was recorded on a UR-20 instrument in paraffin oil and in tablets with KBr. GLC analysis was carried out on a Svet-101 instrument under the conditions described previously [1]. Samples of the glucomannans were hydrolyzed with 1 N H_2SO_4 at 100°C for 8 h. The viscosities of the solutions were measured on an Ostwald viscometer (volume 10 ml) at 28°C.

Fractionation. The precipitation with ethanol of a solution of 1 g of the neutral polysaccharides in 100 ml of water gave fraction 1 (A-2) [1]. Then, with stirring, 50 ml of ethanol was added to the mother solution and the resulting precipitate was separated off (fraction 2). The further addition of ethanol (100 ml) gave fraction 3, and making up the volume of alcohol to fivefold gave fraction 4.

Periodate Oxidation and Smith Degradation of the Glucomannan A-2. A solution of 103 mg of the substance in 100 ml of water was treated with a 0.25 M solution of sodium periodate and the mixture was left in a dark place at 20-22°C. After predetermined intervals of time, 1-ml samples were taken and the excess of sodium periodate was titrated with 0.01 N $Na_2S_2O_3$ and the formic acid with 0.01 N NaOH. The oxidation product was reduced with sodium tetrahydroborate and the polyalcohol was hydrolyzed with 2 ml of 0.5 N H_2SO_4 at 100°C for 8 h. Glycerol and erythritol were detected in the hydrolysate (PC in system 2; revealing agent 2). Part of the hydrolysate was evaporated to dryness and was analyzed by GLC as described by Ovadov [8]. This revealed two peaks corresponding in retention times to the peaks of the acetates of glycerol and erythritol.

Acetylation of the Glucomannan A-2. To 0.1028 g of the substance dried to constant weight were added 30 ml of formamide, 30 ml of pyridine, and, in drops with stirring, 30 ml of acetic anhydride at room temperature over 48 h. Then the reaction mixture was poured into ice water and the precipitate that deposited was separated off, washed with methanol, and dried in vacuum over P_2O_5. Yield 0.16 g.

Oxidation with Chromium Trioxide. The acetate of the glucomannan (0.16 g) was added to a solution of 0.6 g of CrO_3 in 13 ml of glacial acetic acid and the mixture was heated at 50°C for 4 h. Then it was diluted with water and extracted with chloroform, the extract was washed with water and dried over anhydrous sodium sulfate, the chloroform was evaporated off, and the residue was hydrolyzed and subjected to PC in system 1 (revealing agent 1). No hexoses were detected on the chromatogram.

Methylation of the Glucomannan A-2. The glucomannan (1.5 g) was methylated twice by Haworth's method and once by Kuhn's method, and methylation was brought to completion by Purdie's method. This gave a white amorphous powder with a yield of 1.0 g which was readily soluble in acetone, chloroform, and tetrahydrofuran. TLC in system 4 (revealing agent 3) showed the presence of one spot. In the IR spectrum of the glucomannan permethylate the absorption band of hydroxy groups was absent. $[\alpha]_D^{20}$ −20° (c 1.0; chloroform); OCH_3 −40.88%.

Hydrolysis of the Permethylate of Glucomannan A-2. A mixture of 50 mg of the permethylate and 2 ml of 90% formic acid was heated at 100°C for 1 h and was then evaporated to a syrup, which was dissolved in 2.5 ml of 0.25 M H_2SO_4, and this solution was heated at 100°C for 19 h. The hydrolysate was neutralized with $BaCO_3$ and the precipitate was washed with water (2 × 30 ml) and with ethanol (3 × 50 ml) and the resulting solutions were combined and evaporated to a syrup. In this syrup by TLC on Silufol in systems 3 and 5 (revealing agent 1) with markers, 2,3,6-tri-O-mannose, 2,3,6-tri-O-methylglucose, and 2,3,4,6-tetra-O-methylmannose were identified, with R_f 2.2, 3.0, and 5.7, respectively. Part of the hydrolysate was reduced with sodium tetrahydroborate, acetylated, and subjected to GLC. The ratio of 2,3,6-tri-O-methyl-glocose and -mannose found was 1:2.8.

Isolation of 2,3,6-Tri-O-methylmannose. The permethylate of glucomannan A-2 (0.45 g) was subjected to formolysis and hydrolysis by the method described above and was separated on a Silufol plate in system 4. The bands corresponding to 2,3,6-tri-O-methylmannose were eluted with chloroform, and the solvent was evaporated to dryness. Yield 15 mg, $[\alpha]_D^{20}$ −14° (c 1.0; chloroform). The product obtained was found to be identical with the 2,3,6-tri-O-methylmannose isolated by hydrolysis of mannobiose permethylate.

SUMMARY

It has been established by oxidation with chromium trioxide and with sodium periodate and by methylation that the monosaccharide residues in a glucomannan from *Eremurus lactiflorus* O. Fed. are linked by β-1 → 4 bonds and there is a mannose residue at the nonreducing end. The macromolecule of the glucomannan has a linear structure.

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