tetra-O-methyl-D-galactose (115 mg), 3,4-di-O-methyl-L-rhamnose (20 mg), and 2,3,6-tri-O-methyl-D-galactose (980 mg) were obtained. These substances were finally purified by preparative chromatography on paper and were identified in the form of known crystalline derivatives [7, 15].

SUMMARY

The main features of the structure of the pectic acid from the racemes of German chanomile have been established. The main polysaccharide chain consists of D-galacturonic acid residues in the pyranose form with α-1→4 bonds. Isolated single-unit branches of the neutral monosaccharides galactose, arabinose, and xylose are possible. L-rhamnose, which was isolated from the degradation products of the permethylated pectic acid only in the form of 3,4-di-O-methyl-L-rhamnose is apparently included in the main polysaccharide chain.

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THE STRUCTURE OF THE GLUCOMANNAN FROM THE TUBEROUS ROOTS OF Eremurus altaicus

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We have previously described the physicohemical properties of the polysaccharide of fraction I obtained by the fractionation of the initial water-soluble polysaccharide with 96% ethanol [1, 2]. Since the polysaccharide of fraction I is homogeneous, contains only glucose and mannose, and makes up the bulk of the water-soluble polysaccharide, we have investigated the chemical structure of this fraction of the glucomannan.

The glucomannan – a white amorphous powder, viscosity η_{rel} =27.3 (c 0.6%), $[\alpha]_D^{22}$ −41.7° (c 0.6; H_2O) – gives a red coloration with iodine. Gel filtration and sedimentation show that the glucomannan is homogeneous. The ratio of D-glucose and D-mannose in it (GLC) is, as in the purified polysaccharide [1], 1 : 2.6.

The weight-average molecular weight of the glucomannan determined from the sedimentation constant [3] is 120,000 ± 10% which is close to the molecular weight of 108,000 obtained in the gel filtration of the glucomannan through a column of Sephadex G-200 (Fig. 1).

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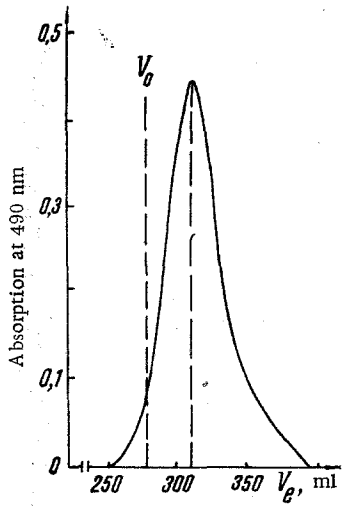

Fig. 1. Gel chromatogram of the glucomannan on Sephadex G-200.

In the plant organism, the glucomannan of Eremurus altaicus is present in partially acetylated form [4]. Treatment of the glucomannan with Fehling's solution and NaOH leads to the formation of regenerated glucomannans which have lost their solubility in water. However, they have the same monosaccharide composition and an identical IR spectrum apart from the absence of the absorption band of ester groups, as in the case of the purified polysaccharide (Fig. 2).

The presence of absorption bands at 1735 and 1250 cm^{-1} in the spectrum of the glucomannan and their disappearance on treatment with solutions of alkalis from the IR spectra of the purified polysaccharide and the regenerated glucomannans and also the results of qualitative and quantitative analysis of the bound acetic acid [5, 6] show that the glucomannan contains O-acetyl (O–Ac) groups that are saponified on treatment with alkalis, and this explains the resulting insolubility in water. Since the products of treatment with Fehling's solution and NaOH are identical, we preferred to treat the glucomannan with a 1% solution of NaOH, and we called the resulting product deactylated glucomannan.

The position of attachment of the O–Ac groups in the glucomannan was determined by the method of Tomoda et al. [7]; 3-O-methyl-D-glucose was found in the products of the hydrolysis of the partially methylated glucomannan (PC, GLC), the formation of this showing that the O–Ac groups in the glucomannan are attached at the C_3 atom of the glucose residue of the repeating section of the chain.

Oxidation of the glucomannan and the deacetylated glucomannan with sodium periodate was complete in 24 h. (Consumption of $NaIO_4$ given in moles per mole of anhydrohexose unit):

Time of taking an aliquot, days	Glucomannan	Deacetylated glucomannan
5	0.7	0.8
9	0.74	0.84
14	0.77	0.9
19	0.8	0.92
24	0.84	0.96
29	0.84	0.96
31	0.84	0.96
HCOOH, mole (31 days)	0.07	0.05

As can be seen from the figures given above, the glucomannan is oxidized incompletely and therefore we subsequently studied the oxidized deacetylated glucomannan.

On Smith degradation [8], erythritol and mannose were found in a ratio of 18 : 1 (GLC), together with traces of glycerol and glucose (PC). The detection of trace amounts of glycerol shows the high molecular weight of the glucomannan, and the considerable amount of erythritol indicates the predominance of a 1→4 bond between glucopyranose and mannopyranose residues.

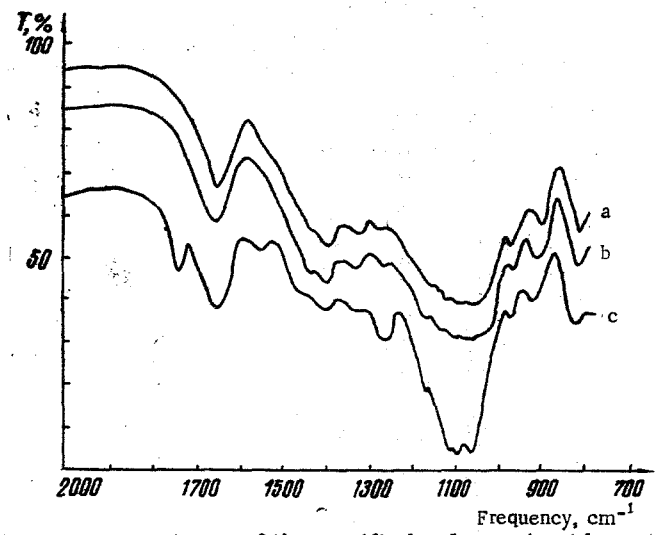

Fig. 2. IR spectrum of the purified polysaccharides (a), the deacetylated glucomannan (b), and the glucomannan (c).

It follows from the amount of formic acid liberated (0.05 mole) and the ratio of erythritol and unoxidized mannose that there is one branching position for every 18-20 hexose residues. In a hydrolyzate of the fully methylated [2] glucomannose, 2,3,4,6-tetra-O-methyl-D-glucose and -D-mannose and 2,3,6-tri-O-methyl-D-glucose and -D-mannose were detected by GLC, TLC with markers, and mass spectroscopy [9, 10]. The bulk of the hydrolyzate consisted of the tri-O-methylhexoses, which were present in them in a ratio of 1 : 2.3 (GLC). Because of their very small amount, the di-O-methylhexoses could not be identified.

The sequence of decomposition of the carbohydrate residues in the glucomannan chain was determined by partial acid hydrolysis and acetolysis. The mixture of mono- and oligosaccharides obtained was fractionated first by chromatography on a column of carbon–Celite and then by preparative PC separation.

The products of partial acid hydrolysis and acetolysis contained mannose, glucose, and about 10 oligosaccharides (A-F) were isolated in the pure form. The structures of oligosaccharides A, B, C, and D have been reported previously [2]. A substance with R_m 0.38 was found previously between the spots of the oligosaccharides C and D, but its structure was not determined because of its insufficient amount. In the acetolysis products, unlike the products of partial hydrolysis, the amount of oligosaccharides was quantitatively predominant. We isolated oligosaccharides with R_m 0.38 (C_1), 0.25 (E), and 0.15 (F). The structures of the oligosaccharides were determined by complete and partial acid hydrolyses before and after reduction with sodium tetrahydroborate and periodate oxidation.

On hydrolysis, oligosaccharide C_1 gave D-glucose and D-mannose (1:2). Partial hydrolysis of the reduced oligosaccharide led to D-glucose, D-mannose, and oligosaccharide C, and the products of complete acid hydrolysis were D-glucose and D-mannose (1 : 1). Consequently, the oligosaccharide has a reducing end consisting of D-mannose. Periodate oxidation of the oligosaccharide followed by Smith degradation led to the formation of glycerol and erythritol (1 : 2). The oligosaccharide has a degree of polymerization of 3. It was identified as O-β-D-mannopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-D-mannose.

On complete hydrolysis, oligosaccharide E gave glucose and mannose (1:2). The products of the partial acid hydrolysis of the reduced oligosaccharide were found to contain D-mannose and oligosaccharide B. This shows that oligosaccharide E has D-glucose at the reducing end.

In the products of Smith degradation there were glycerol and eryrthritol (1 : 2), and this oligosaccharide is therefore O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-D-glucose.

On complete acid hydrolysis, oligosaccharide F was split into glucose and mannose (1 : 3), and on partial hydrolysis it gave oligosaccharides A, B, C, C_1, and E, in addition to mannose and glucose. Complete hydrolysis of the reduced oligosaccharide gave D-glucose and D-mannose (1 : 2), and partial hydrolysis gave oligosaccharides B, C, and E. The reducing end of the oligosaccharide is D-mannose. Glycerol and erythritol were found in the products of Smith degradation in a ratio of 1 : 3, which shows the presence of a linear 1→4-linked chain in the oligosaccharide. On the basis of the facts given, for oligosaccharide F we propose the tetrasaccharide structure O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→4)-D-mannose.

No mannotetraose was found in the products of partial acid hydrolysis and acetolysis, as also follows from the ratio of the components (1 : 2.6). The absence of cellobiose permits the conclusion that there are no glucose residues attached to one another in the glucomannan chain.

Thus, on the basis of the results of periodate oxidation, methylation, partial cleavage of the glycosidic bonds, and IR spectroscopy, it may be assumed that the chain of the glucomannan contains repeating sections consisting of β-1→4-linked residues of D-glucopyranose and D-mannopyranose in a ratio of 1 : 2.6 and has one of the following structures:

1.-[-Glcp-(1→4)-O-β-*D*-Manp-(1→4)-O-β-*D*-Manp-(1→4)-O-β-*D*-Glcp-(1→4)-O-β-*D*-Manp-(1→4)-O-β-*D*-Manp-(1→4)-O-β-*D*-Manp-]$_n$-;

2.-[-Manp-Glcp-Manp-Manp-Manp-Glcp-Manp-]$_n$-;

3.-[-Manp-Manp-Glcp-Manp-Manp-Manp-Glcp-]$_n$-, where $n = 95 - 100$.

EXPERIMENTAL

Solutions were evaporated in a rotary evaporator at 40 ± 5°C, and specific rations were measured at 22 ± 2°C. Paper chromatography (PC) was performed on Filtrak-7, 12, and 14 papers (GDR) by the descending and ascending methods using the following solvent systems (by volume): 1) butan-1-ol–pyridine–water (6 : 4 : 3); 2) ethyl acetate–pyridine–water (7 : 2 : 1); 3) propan-1-ol–ethyl acetate–water (7 : 2 : 1).

Thin-layer chromatography (TLC) was performed on plates with a layer of type KSK silica gel in systems 4) methyl ethyl ketone–1% aqueous ammonia (30 : 4) and 5) benzene–acetone–water (5 : 5 : 1).

The following reagents were used for indicating the spots: 1) aniline hydrogen phthalate (10-15 min at 105-110°C) [11], and 2) periodate–$KMnO_4$–benzidine [11].

Gas–liquid chromatograms of the samples were recorded on a Tsvet-101 instrument with a flame-ionization detector under the following conditions:

A – steel column (0.3 × 200 cm); Chromaton N-AW 0.200-0.250 mm, impregnated with 5% of Silicone XE-60; temperature 210°C; air 300, H_2 35, N_2 35 ml/min.

B – steel column (0.3 × 100 cm); Chromaton N-AW, 0.100-0.125 mm, HMDS 5% + 1% PEGA; temperature 50°C; N_2 30 ml/min.

C – steel column (0.3 × 200 cm), Chromaton N-AW-DMSS 5%, SE-30; temperature 180°C; He 25 ml/min.

The oligosaccharides were hydrolyzed by heating a 10-mg sample in 1 ml of 1 N H_2SO_4 on the boiling water bath for 2-4 h, and then the hydrolyzates were neutralized with $BaCO_3$, filtered, deionized with Amberlite IR-120 (H^+), evaporated, and chromatographed in systems 1 and 2. A solution of 10 mg of the oligosaccharide in 5 ml of water was reduced with 20 mg of sodium tetrahydroborate overnight, 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 product was hydrolyzed under similar conditions.

The partial acid hydrolysis of an oligosaccharide and the product of its reduction was performed by heating 10 mg of sample in 2 ml of 0.1 N H_2SO_4 on the boiling water bath. The hydrolyzate was treated by the method described above.

The degrees of polymerization of oligosaccharides C_1, E, and F were determined by gas–liquid chromatography from the ratio of reducing sugars before and after the reduction of the oligosaccharide with sodium tetrahydroborate.

Oxidation of the Oligosaccharides. A mixture of 10 mg of the specimen and 10 ml of 0.05 M $NaIO_4$ was stirred in the dark at +5°C. The consumption of sodium periodate was determined by titration with 0.01 N $Na_2S_2O_3$. The oxidized oligosaccharide was subjected to Smith degradation, and the product was analyzed by PC in systems 2 and 3 with the use of reagents 1 and 2 to reveal the spots. The GLC of the product was performed by the method of Buchala et al. [12] (conditions A).

The mobilities of the oligosaccharides on paper chromatograms are given in relation to the mobility of D-mannose (R_m).

Gel Chromatography of the Glucomannan. A solution of 30 g of the glucomannan in 3 ml of 0.3% NaCl was deposited on a column (3.2 × 100 cm) filled with Sephadex G-200. Elution was performed with the same solu-

tion. The eluates were collected in 3-ml fractions, and these were analyzed by the phenol/sulfuric acid method [13]: V_e =308.5 ml. The column was calibrated with dextrans having molecular weights of 110,000 (V_e =308 ml), 80,000 (V_e =324 ml), and 40,000 (V_e =402 ml); V_0 =275.5 ml.

The molecular weight of the glucomannan was determined from a calibration curve expressing the dependence of the volume of eluent V_e on the logarithm of M_n.

The ultracentrifugation of a 1% aqueous solution of the glucomannan was performed on an MOM-3170 instrument at 50,000 rpm, temperature 20°C, rate of recording 5 min.

Determination of the O–Ac Groups in the Glucomannan. a) The O–Ac groups were identified by Meier's method [14]. GLC (conditions B) showed one peak corresponding in its retention time to the methyl acetate peak.

b) Quantitative analysis of the O–Ac groups was performed by a handbook method [6]; 4.4% of O–Ac groups was found.

Determination of the Position of Attachment of the O–Ac Groups. The glucomannan (2 g) was methylated by the method of Tomoda et al. [7], and the resulting partially methylated glucomannan was hydrolyzed with 4 ml of 2 N H_2SO_4 for 16 h, the hydrolyzate being worked up in the usual way. PC in systems 1 and 2 showed the presence of glucose and mannose and a spot with R_f 0.53, which was isolated by preparative PC (system 1) and identified (PC) as 3-0-methyl-D-glucose, a sample of which we synthesized by a known method [15, 16]. The substance with R_f 0.53 was demethylated by Wadman's method [17] and the product was identified chromatographically (PC, systems 1 and 2) as D-glucose.

Saponification of the Glucomannan by Fehling's Solution. A solution of 1 g of the glucomannan in 300 ml of water was precipitated with Fehling's solution as in the case of the purified polysaccharide [1]. This gave a white powder with a yield of 82.5%. IR spectrum: 820, 890, 1520, 1650, 3200-3400 cm^{-1}.

Saponification with NaOH Solution. A solution of 1 g of the glucomannan in 90 ml of water was treated with 10 ml of 10% NaOH (final concentration 1%), the mixture was stirred overnight and was precipitated with three volumes of ethanol, the precipitate was separated off and dispersed in one liter of water, the medium was acidified to pH 3, and the precipitate was separated off by centrifuging, washed with water to neutrality, and dried with methanol, acetone, and ether, and over P_2O_5. Yield 79.0%. Its IR spectrum was similar to that of the substance obtained in the saponification of the glucomannan by Fehling's solution.

Periodate Oxidation. The deactylated glucomannan (0.5025 g; 0.0031 mole) was oxidized with a 0.03 M solution of $NaIO_4$ at +5°C. Aliquots (1 ml) were taken after predetermined intervals of time and the excess of $NaIO_4$ was titrated with $Na_2S_2O_3$, and the formic acid produced with 0.01 N NaOH.

Smith Degradation. The oxidized deacetylated glucomannan was reduced with sodium tetrahydroborate, and the resulting polyalcohol (yield 0.13 g) was hydrolyzed with 4 ml of 0.5 N H_2SO_4 at 100°C for 8 h. The hydrolyzate was found to contain traces of glycerol and erythritol (PC, system 3, revealing agent 2), and traces of glucose and mannose (PC, system 1, revealing agent 1). Part of the hydrolyzate was evaporated to dryness and analyzed by GLC (condition A) by a handbook method [18]. Three peaks were found corresponding in their retention times to the peaks of glycerol triacetate, erythritol tetraacetate, and D-mannose aldononitrile acetate.

Methylation. The glucomannan acetate (6 g) was methylated once by the modified Haworth method [16] and twice by Hakomori's method [20]. This gave the fully methylated glucomannan with a yield of 0.34 g; OCH_3–41.2%, $[\alpha]_D$–16.6°, (c 0.9; $CHCl_3$). IR spectrum: the hydroxyl absorption band was absent.

Hydrolysis. Hydrolysis of the methylated glucomannan (0.1 g) was performed by the method of Bouveng et al. [21]. The product was analyzed by TLC in systems 4 and 5 (revealing agent 1) and by GLC under conditions A and B.

Partial Hydrolysis. a) The deacetylated glucomannan (5.5 g) was treated with 140 ml of 90% HCOOH for 1.5 h, the reaction mixture was diluted with 140 ml of water and was heated on the boiling water bath for 2.5 h and was then evaporated to dryness. The residue was hydrolyzed with 60 ml of 0.5 N H_2SO_4 for 10 min. A syrup was obtained (7.12 g); PC in systems 1 and 2 showed the presence of mannose and glucose and of spots with R_m 0.8; 0.61; 0.48; 0.38; 0.32; 0.25; 0.15; 0.10; 0.05; 0.03.

b). Acetolysis. The acetylated glucomannan (7.5 g) was subjected to acetolysis by the method of Kato et al. [22]. This gave 4.1 g of a syrup showing on PC in systems 1 and 2 the same spots as in experiment a).

Separation of the Products of Partial Acid Hydrolysis. A solution of 7.12 g of the syrup in 20 ml of water was deposited on a column (3 × 33 cm) filled with a mixture of carbon and Celite-535 (1 : 1). On elution with water, fraction I (3.5 liters) was found to contain mannose and glucose, and fraction II (15.5 liters) traces of mannose and of glucose and the oligosaccharide B, and fraction III (16 liters) contained oligosaccharide B. Eluting the column with 2.5% ethanol (3.5 liters) gave fraction IV containing oligosaccharides A and B. Fraction V (A, C, C_1, and E) and VI (A, C, C_1, D, E, F, G, H, and I) were obtained by eluting the column with 5% ethanol (2.5 liters and 7.5% ethanol (11 liters), respectively. Fractions IV-VI were then separated by preparative PC in a system 1, and individual oligosaccharides were obtained.

SUMMARY

By fractionating the initial water-soluble polysaccharide from the tuberous roots of Eremurus altaicus, a homogeneous naturally acetylated glucomannan with a molecular weight of 108,000-120,000 has been isolated.

On the basis of the results of periodate oxidation and methylation and a study of the products of partial cleavage of the glycosidic bonds, the structure of a repeating heptasaccharide section of the chain consisting of β-1→4-linked D-glucopyranose (2 moles) and D-mannopyranose (5 moles) residues has been established. The glucomannan apparently contains 95-100 such sections. It has been shown that in each repeating solution there is one O-acetyl group at a C_3 atom of a glucose residue.

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