POLYSACCHARIDES OF *Ungernia*.

VI. A MANNAN FROM THE BULBS OF *Ungernia vvedenskyi*

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The results of a study of the structure of ungeromannan-V from *Ungernia vvedenskii* Khamidkh by oxidation with chromic oxide and with sodium periodate and by methylation have shown that the monosaccharide residues in it are linked by β-1 → 4 glycosidic bonds. The macromolecule of ungeromannan-V has slight branching.

Plants of the genus *Ungernia* Bge. are rich in water-soluble polysaccharides [1, 2] the bulk of which is composed of mannans [1, 3]. In the present paper we give the results of a study of a mannan from the bulbs of *U. vvedenskyi* Khamidkh [4].

The air-dry raw material was inactivated with ethanol and polysaccharides were extracted from the residue of the raw material with water at room temperature by the method described previously [1]. This gave a water-soluble polysaccharide (PS) with a yield of 11%, and in a hydrolysate of this by PC we detected rhamnose, xylose, arabinose, mannose, glucose, and galactose, and traces of galacturonic acid. This showed that the PS isolated was a mixture of neutral and acidic polysaccharides. We therefore separated them on a column of DEAE-cellulose (acetate form). The neutral polysaccharide (NPS), elutable by water, amounted to 68%, and in its hydrolysate we found arabinose, mannose (main component), and glucose. The polysaccharides elutable by 1 M sodium acetate amounted to 12% and consisted of a mixture of neutral monosaccharide and of galacturonic acid.

Gel chromatography of the NPS on a column of Sephadex G-150 showed its polydispersity. To obtain a homogeneous polysaccharide it was fractionally precipitated with ethanol from aqueous solution. This gave four fractions with yields of 26, 56, 8, and 3%, respectively, of which fraction II was obtained in the highest yield. In a hydrolysate of this, arabinose, mannose, and glucose were identified by PC and GLC in a ratio of 1:79:2. According to the results of sedimentation analysis [5] and gel chromatography on Sephadex G-150, fraction II was homogeneous. We have called it ungeromannan-V and have studied its structure.

Ungeromannan-V is a white amorphous powder which dissolves in water to form a colloidal solution with $[\alpha]_D^{25}$ −37° (c 1.0; water) and a relative viscosity of 55.3 (c 1.0; water); it gives no coloration with iodine. The molecular weight, determined from the sedimentation constant [5], is 100,000 (±10%), which is close to that obtained by gel chromatography on a column of Sephadex G-150 (105,000).

In the plant organism, ungeromannan-V exists in partially acetylated form. This is confirmed by the results of qualitative [6] and quantitative [7] analyses for the content of O-acetyl groups (5%) and also by the presence of absorption bands in the IR spectra at 1240 cm^{-1} (C—O stretching vibrations of ester groups) and 1750 cm^{-1} (CO stretching vibrations of carbonyl groups). When the ungeromannan was treated with Fehling's solutions or alkali, the O-acetyl groups underwent saponification (absence of the above-mentioned absorption bands) and the substance lost its solubility in water. In this procedure the ratio of the monosaccharides remained as before (1:78:2). This corresponds to information in the literature. Thus, Misaki et al. [8] detected an acetyl group on every other D-mannose residue in the mannan they isolated.

The periodate oxidation of the deacetylated ungeromannan-V (DAU) was complete in ten days. The consumption of sodium periodate amounted to 0.95 mole per mole of anhydrohexose unit, and the amount of formic acid liberated was 0.03 mole.

*Deceased.

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On Smith degradation [9], PC showed the presence of erythritol as the main product, and also traces of glycerol and mannose, while GLC showed only erythritol. The detection of only trace amounts of glycerol indicates a high molecular weight of the ungeromannan-V, and the considerable amount of erythritol indicates the presence of a 1 → 4 bond between the hexopyranose residues.

Methylation of ungeromannan-V by Hakomori's method [10] yielded a permethylate the IR spectrum of which lacked the absorption band of a hydroxy group. In a hydrolysate of it, TLC with markers showed the presence of 2,3,6-tri-O-methyl-D-mannose, 2,3,6-tri-O-methyl-D-glucose, 2,3,4,6-tetra-O-methyl-D-mannose, and di-O-methylhexoses, the last three being represented in trace amounts. The isolation of 2,3,6-tri-O-methyl-D-mannose as the main component of the hydrolysate of the permethylate shows the presence of a 1 → 4 bond in ungeromannan-V.

The negative specific rotation of the ungeromannan-V and of its permethylate, and an absorption band at 890 cm^{-1} in the IR spectrum show the presence of a β-glycosidic link between the monosaccharide residues. To confirm this assumption, we used a method based on the oxidation of the fully acetylated polysaccharide with chromium trioxide in glacial acetic acid [11]. Analysis of the reaction product showed that all the sugar residues had undergone oxidation (there were no monosaccharides in the hydrolysate); this demonstrates the presence of a β-glycosidic bond. In the case of an α-glucosidic bond, oxidation would not have taken place and free sugars would have been found in the reaction product.

Thus, a β-1 → 4 glycosidic bond has been established in the ungeromannan-V molecule. The detection of trace amounts of mannose in the case of periodate oxidation and of a di-O-methylhexose in a hydrolysate of the permethylate indicates slight branching.

EXPERIMENTAL

Solutions were evaporated at 40 ± 5°C. Paper chromatography was performed on Filtrak-FN 7, 12, and 14 papers by the descending method using the following solvent systems: 1) butan-1-ol—pyridine—water (6:4:3); 2) propan-1-ol—ethyl acetate—water (7:2:1). Thin-layer chromatography (TLC) was performed on Silufol plates in the following systems: 3) methylethyl ketone—1% aqueous ammonia (30:4); and 4) benzene—acetone—water (5:5:1). The following reagents were used to indicate the spots: 1) aniline hydrogen phthalate (10-15 min at 105-110°C) [12]; and 2) periodate—$KMnO_4$—benzidine [12]. The GLC of the samples was performed on a Tsvet-101 instrument with a flame-ionization detector under the following conditions: stainless-steel column (200 × 0.3 cm) 5% of silicone XE-60 on Chromaton NAW, 0.200-0.250 mm 210°C; carrier gas helium, 60 ml/min. Acetates of aldononitriles were obtained as described by Ovodov [13] and so were acetates of polyols of partially methylated sugars.

Isolation of the Polysaccharides. A mixture of 100 g of air-dry comminuted raw material and 500 ml of ethanol was boiled for 1 h and filtered. The residue of the raw material was dried and was extracted with water at room temperature three times (1.5, 1, and 1 liter) for 1 h each time. The extracts were combined and were treated with a mixture of chloroform and amyl alcohol, and the precipitate of proteins was separated by centrifuging; then the solution was evaporated to 800 ml and the polysaccharides were precipitated with 2.5 liters of methanol and were washed with acetone and ether and dried in vacuum over P_2O_5; yield 11.0 g.

Hydrolysis of the Polysaccharide. A mixture of 50 mg of the substance and 2.5 ml of 1 N H_2SO_4 was heated on the boiling water bath for 18 h and was then neutralized with barium carbonate and deionized with KU-2 cation-exchange resin, after which PC (systems 1 and 2, revealing agent 1) showed the presence of galacturonic acid and neutral monosaccharides: rhamnose, xylose, arabinose, mannose, glucose, and galactose.

Chromatography on DEAE-Cellulose. DEAE-Cellulose (bead polymer) (100 g) was treated successively with 0.5 N NaOH and 0.5 N HCl and then with a 1-M solution of CH_3COONa, and it was placed in a column (4.5 × 40 cm), being washed each time to neutrality. On the DEAE-cellulose so obtained was deposited 100 ml of a 1% solution of the polysaccharide, and elution was carried out with 1 liter of water and 1 liter of 1 M sodium acetate. The aqueous eluates were evaporated to 50 ml and were precipitated with ethanol (1:5). Yield 0.68 g (NPS). In a hydrolysate arabinose, mannose, and glucose were detected by PC (system 2, revealing agent 1). The polysaccharides eluted by 1 M sodium acetate were dialized against distilled water, evaporated to 20 ml, and treated with methanol (1:5). This gave 0.12 g of an acid polysaccharide in a hydrolysate of which galacturonic acid and the neutral monosaccharides

rhamnose, xylose, arabinose, mannose, glucose, and galactose were detected by PC (system 2, revealing agent 1).

Gel Filtration of the Neutral Polysaccharide. A solution of 10 mg of the substance in 1 ml of 0.3% NaCl was deposited on a column (2 × 40 m) of Sephadex G-150. Elution was performed with the same solvent. The eluates were colleted in 3-ml portions, and these were analyzed by the phenol–sulfuric acid method [14]. Peaks were obtained with V_{e1} = 53 ml and V_{e2} = 60 ml.

Fractionation of the NPS. A solution of 1 g of the neutral polysaccharide in 200 ml of water was treated dropwise with 200 ml of ethanol with vigorous stirring. The precipitate that deposited was separated off by centrifuging and was dried. Yield 0.26 g (fraction I). Another 75 ml of ethanol was added to the supernatant liquid, and the precipitate was separated off similarly. Yield 0.56 g (fraction II), ungeromannan-V. Fraction III was isolated by the addition of 100 ml of ethanol. Yield 0.08 g. The aqueous ethanolic solutions were evaporated and were precipitated with three volumes of ethanol. Yield 0.03 g (fraction IV).

Hydrolysis of Ungeromannan-V. A mixture of 50 mg of the substance with 2.5 ml of 1 N H_2SO_4 was heated on the water bath for 18 h and was then neutralized and deionized, and PC (systems 1 and 2, revealing agent 1) showed the presence of arabinose, mannose, and glucose. Their ratio, according to GLC (of the corresponding aldononitrile acetates) was 1:79:2.

The hydrolysate was treated with phenylhydrazine by the method of Usov et al. [15]. This gave D-mannose phenylhydrazone with mp 189-190°C; according to the literature [16], mp 188-190°C.

Gel Filtration of Ungeromannan-V. A solution of 10 mg of the substance of 1 ml of 0.3% NaCl was deposited on a column of Sephadex G-150. Analysis was carried out as described above: V_e = 42.8 ml, the column being calibrated with dextrans having molecular weights of 110,000 (V_e = 43.7 ml) and 80,000 (V_e = 47.3 ml). The molecular weight of the ungeromannan-V was determined from a calibration curve expressing the dependence of the elution volume V_e on the logarithm of M_n.

Determination of the O-Acetyl Groups in Ungeromannan-V. A. Qualitative analysis was performed by the method of Kuznetsov and Stepanenko [6] by the preparation of acetohydroxamic acid, the chromatographic mobility of which coincided with that of a standard, R_f 0.61. B. For quantitative determination, 0.096 g of the substance was dissolved in 25 ml of water, 25 ml of 1 N NaOH was added, and the mixture was stirred for 8 h and was then titrated with 0.5 N HCl (with phenolphthalein as indicator). A blank experiment was performed in parallel. Found: O–Ac groups 5%.

Deacetylation of Ungeromannan-V. A solution of 100 mg of the substance in 50 ml of water was treated, with stirring, with 1.5 ml of Fehling's solution. The precipitate was separated off by centrifuging and was washed successively with 80% and 5% solutions of acetic acid and then with water and was dehydrated with ethanol. Yield 80 mg (DAU).

In the cold, 50 mg of DAU was dissolved in 2 ml of 72% sulfuric acid, and then the concentration was brought to 2 N and the solution was heated at 100°C for 16 h. The hydrolysate was neutralized and deionized, and PC (system 1, revealing agent 1) showed the presence of arabinose, mannose, and glucose, their ratio being found by GLC to be 1:78:2.

Periodate Oxidation and Smith Degradation of Ungeromannan-V. A suspension of 100 mg of DAU in 100 ml of water was treated with 15 ml of a 0.25 M solution of sodium periodate and it was left in the dark at room temperature (15°C). Samples with a volume of 1 ml were taken every day and the excess of sodium periodate was titrated with 0.01 N sodium thiosulfate solution. After 10 days, the consumption of sodium periodate amounted to 0.95 mole and it did not change further. The formic acid liberated was titrated with 0.01 N NaOH, which showed the presence of 0.03 mole. The oxidation product was reduced with sodium tetrahydroborate and the resulting product was hydrolyzed with 5 ml of 0.5 N H_2SO_4 for 8 h at 100°C, after which PC showed the presence of traces of mannose (system 1, revealing agent 1), and also of erythritol and traces of glycerol (system 1, revealing agent 2). Part of the hydrolysate was analyze by GLC [13]. This showed a peak corresponding in retention time to that of erythritol acetate.

Methylation of Ungeromannan-V. The substance (0.46 g) was methylated twice by Hakomori's method [10]. This gave a fully methylated product with a yield of 0.41 g; OCH_3 40.7%, $[\alpha]_D^{22}$ –22° (c 1.0; $CHCl_3$); IR spectrum: hydroxyl absorption band absent. Hydrolysis of the per-

methylate was carried out by the method of Bouveng et al. [17]. The following were detected by TLC (systems 3 and 4, revealing agent 1): 2,3,4,6-tetra-O-methyl-D-mannose, 2,3,6-tri-O-methyl-D-glucose, 2,3,6-tri-O-methyl-D-mannose, and a di-O-methylhexose with R_f 0.75, 0.52, 0.4, and 0.15, respectively. GLC showed that the 2,3,6-tri-O-methyl-D-mannose was the main component.

Oxidation of Ungeromannan-V with Chromium Trioxide. With stirring, 0.2 g of the substance was dissolved in 40 ml of formamide, and 40 ml of pyridine and 30 ml of acetic anhydride were added. The mixture was stirred for 5 days and was then poured into 2 liters of ice water. The precipitate was centrifuged off, washed with water, and dried over P_2O_5. Yield 0.25 g. IR spectrum: hydroxy absorption band absent; bands 1240 and 1250 cm^{-1} well defined. The amount of O-acetyl groups [7] was 43%. To a solution of 0.8 g of CrO_3 in 15 ml of glacial acetic acid was added 0.2 g of the acetate of ungeromannan-V and the mixture was heated at 50°C for 4 h. Then it was diluted with water and extracted with chloroform, and the chloroform extract was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was dissolved in 1 ml of 1 N H_2SO_4 and was hydrolyzed on the boiling water bath for 16 h, after which the hydrolysate was worked up by the usual method. PC (systems 1 and 2, revealing agent 1) revealed no hexose.

SUMMARY

1. It has been established that the bulbs of *Ungernia vvedenskyi* **Khamidkh contain 11%** of water-soluble polysaccharides. Ungeromannan-V, which is acetylated in the native state, was isolated by fractionation of the netural polysaccharides.

2. On the basis of the results of periodate and chromic oxidation and of methylation, it has been shown that in ungeromannan-V the D-mannose residues are attached by β-1 → 4 glycosidic bonds and its molecule has a linear structure with slight branching.

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