POLYSACCHARIDES OF Ungernia.

XIV. A MANNAN FROM Ungernia trisphaera BULBS

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The group carbohydrate composition of the bulbs of Ungernia trisphaera has been studied. The homogeneous ungeromannan-Tr, acetylated in the native state, has been isolated. On the basis of the results of periodate oxidation, methylation, partial hydrolysis, and IR and ^{13}C NMR spectroscopy, it has been shown that ungeromannan-Tr consists of a linear polymer D-mannopyranose with β-(1 → 4)-glycosidic bonds.

Continuing an investigation of the polysaccharides of plants of the genus Ungernia [1-3], we have studied the carbohydrate composition of bulbs of U. trisphaera Bunge collected in the vegetation phase on May 7, 1983, in the village of Babadurmez, Turkmen SSR.

From one sample of the raw material previously treated with 96% ethanol, the ethanol-soluble substances (ESSs), the water-soluble polysaccharides (WSPSs), the pectin substances (PSs), and hemicelluloses (HCs) A and B were extracted successively. The amounts of the carbohydrate components and their monosaccharide compositions are given below:

Type of carbohydrate	Yield, %	Monosaccharide composition						
		GalUA	*Gal*	*Glc*	*Ara*	*Man*	*Xyl*	*Rha*
EESs	18,0	—	—	+	—	—	—	—
WSPSs	8,2	Tr.	1,4	1	1	51,3	—	—
PS	7,0	+	1,5	25,4	—	Ca.	1	1,3
HC-A	2,6	+	—	25,2	1	1,5	—	1,6
HC-B	4,5	Tr.	—	42	Tr.	1,5	1	—

With a 0.1 N solution of iodine, the aqueous solutions of the pectin substances and of the hemicelluloses gave a blue coloration and therefore they included glucans of the starch type, which also explains the high level of glucose in the PSs, HC-A, and HC-B.

The WSPSs were studied in more detail. They contained galacturonic acid, which means that they consisted of a mixture of neutral and acidic polysaccharides. In order to obtain the neutral polysaccharide (NPS), the WSPSs were separated on a column of DEAE-cellulose (acetate form). The NPS was eluted with water, its yield amounting to 60% of the WSPSs. A hydrolysate was found to contain very small amounts of glucose and galactose, with mannose as the main component. The acidic polysaccharides were eluted with a 0.1 N solution of NaOH in a yield of 20%. A hydrolysate of them was found to contain galacturonic acid, galactose, glucose, and mannose.

Gel chromatography of the NPS on a column of Sephadex G-100 showed its polydispersity. In order to obtain a homogeneous mannan, the NPS was fractionally precipitated with ethanol from aqueous solution. Three fractions were obtained (20, 30, and 35%, respectively), in the first of which mannose predominated, with trace amounts of glucose. The predominance of mannose in the polysaccharides showed that they belong to the class of mannans. On this basis, fraction 1 was called ungeromannan-Tr.

Gel chromatography showed the homogeneity of the mannan under investigation and a molecular weight for it of 90,000.

Ungeromannan-Tr consisted of a white amorphous powder dissolving slowly in water with the formation of a viscous solution, the relative viscosity of a 1% solution being 14.1; $[\alpha]D^{20}$ −34° (c 1.0; water), which indicated the β configuration of the link between the D-mannoses. Ungeromannan-Tr consisted of a partially acetylated polysaccharide, as was con-

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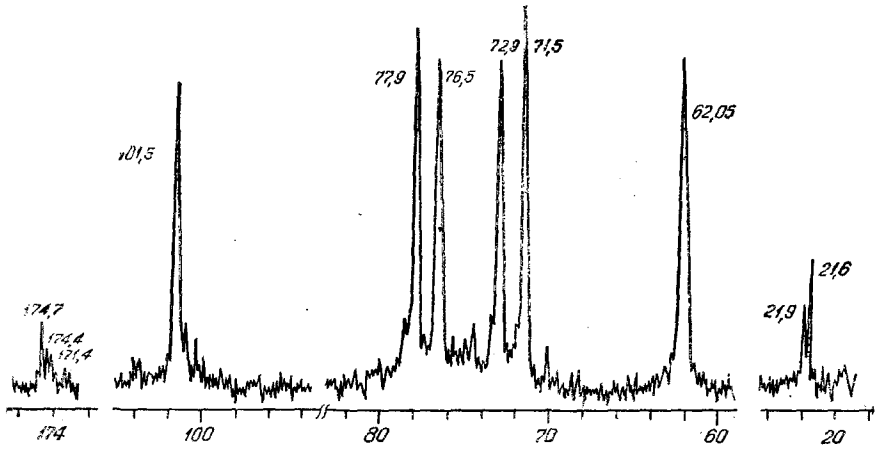

Fig. 1. ^{13}C NMR spectrum of ungeromannan-Tr.

firmed by the results of quantitative analysis for O-Ac groups (4.5%) [4] and also by the presence of absorption bands in the IR spectrum at 1240 and 1750 cm^{-1}. The O—Ac groups were readily saponified with Fehling's solution or caustic soda, which led to the formation of a deacetylated ungeromannan (DAU) in the IR spectrum of which the absorption bands mentioned were absent.

The structure of the mannan was established by the methods of periodate oxidation and methylation, and also by partial hydrolysis and ^{13}C NMR spectroscopy.

Ungeromannan-Tr was oxidized with sodium periodate. Erythritol and trace amounts of glycerol were found in the products of Smith degradation, which indicated the presence of a (1 → 4) bond and a linear arrangement of the D-mannose residues.

The Hakomori methylation [5] of ungeromannan-Tr gave a permethylate the IR spectrum of which lacked the absorption band of a hydroxy group. The permethylate was subjected to formolysis and hydrolysis, and the main product detected in the hydrolysate by TLC was 2,3,6-tri-O-methyl-D-mannose, together with trace amounts of 2,3,4,6-tetra-O-methyl-D-mannose and 2,3,6-tri-O-methyl-D-glucose. The presence of 2,3,6-tri-O-methyl-D-mannose as the main product of the permethylate showed a (1 → 4) bond between the monosaccharide residues and confirmed the linear nature of the mannan chain.

When the DAU was subjected to partial hydrolysis with formic acid [6], PC revealed mannose and oligosaccharides with Rf values relative to mannose of 0.11, 0.28, and 0.6. The oligosaccharides were identified with markers as mannotetraose, mannotriose, and mannobiose.

The mannans isolated from the Ungernia plant have not previously been studied by the ^{13}C NMR spectroscopic method. An interpretation of the spectra of the mannans will permit their characteristic features to be determined, which is important in the study of a large series of monotypical polysaccharides. In this case, it is sufficient to investigate the structure of one polysaccharide and to deduce the structures of the others by comparing their spectra.

Ungeromannan-Tr formed viscous solutions which impaired the resolution of the ^{13}C NMR spectra. For study we therefore used a partially destructured mannan obtained by the hydrolysis of the initial ungeromannan-Tr. Its spectrum is shown in Fig. 1.

In the region of signals corresponding to the glycosidic C_1 atoms there is a signal at 101.5 ppm corresponding to a β-(1 → 4) bond between D-mannose residues. A signal at 77.9 ppm corresponds only to C_4 of a β-(1 → 4)-bound mannan. The remaining signals corresponding to mannose residues can be assigned on the basis of literature information [7, 8], to C_2 (71.5 ppm), C_3 (72.95 ppm), C_5 (76.5 ppm), and C_6 (62.05 ppm). The presence of acetic acid residues is shown by signals with chemical shifts of 21.6-21.9 and 174.1-174.7 ppm.

Thus, the ^{13}C NMR spectrum that was obtained, on the whole, completely confirmed the structure of the acetylated mannan that had been established chemically. It may be concluded that the ungeromannan-Tr under study was a high-molecular polysaccharide acetylated in the native state that was based on a linear carbohydrate chain of β-(1 → 4)-bound D-mannopyranose residues.

The results of a study of the WSPSs of U. trisphaera and of other species of Ungernia [2, 3] have shown that the presence of partially acetylated (1 → 4)-bound mannans is characteristic for the bulbs of plants of the genus Ungernia.

EXPERIMENTAL

Monosaccharides were chromatographed on Filtrak FN-7,11,12 paper by the descending method using the solvent system 1) butan-1-ol—pyridine—water (6:4:3). Thin-layer chromatography was performed on Silufol plates in systems 2) methyl ethyl ketone—1% ammonia (30:4) and 3) benzene—acetone—water (5:5:1). The indication of the spots was achieved with the aid of the following reagents: 1) aniline hydrogen phthalate and, 2) periodate —$KMnO_4$—benzidine [9]. The GLC of the samples in the form of the acetates of the corresponding aldonitriles was performed on a Chrom-5 instrument using a steel column (0.4 × 100 cm) filled with Chromaton (0.16-0.20 mesh) impregnated with 5% of XE-60 at a temperature of 210° and a rate of flow of helium of 60 ml/min. A flame-ionization detector was used. The derivatives were obtained as described by Ovodov [10]. The ^{13}C NMR spectrum was obtained on a Bruker WM-250 spectrometer using a 3% solution of the polysaccharide in D_2O at 80°C with CH_3OH as internal standard (50.15 ppm).

Isolation of the Ethanol-Soluble Substances. The air-dry comminuted raw material (50 g) was twice boiled with 200 ml of ethanol for 1 h. The dried raw material was extracted three times with 82% ethanol using 100 ml for 30 min each time. The ethanolic extracts were evaporated to 50 ml and were treated successively with a saturated solution of lead acetate and sodium sulfate to eliminate low-molecular-weight compounds. Then it was evaporated again to the state of a syrup and this was chromatographed on paper (system 1, revealing agent 1). No free monosaccharides were detected. A mixture of 0.1 g of the syrup and 3 ml of 0.5 N sulfuric acid was treated at 100°C for 2 h. The resulting hydrolysate was treated with barium carbonate and with KU-2 cation-exchange resin (H^+) and was chromatographed under the same conditions. Only glucose was detected.

Isolation of the WSPSs. The residue of raw material after the elimination of the ESSs was extracted with water (2 × 100 and 1 × 100 ml) and was separated from proteins, after which a mixture of chloroform and butanol (100 and 20 ml per 500 ml of extract) was added, the mixture was stirred for 30 min, the precipitate was separated off by centrifugation, and the solution was evaporated and precipitated with acetone (1:4). The precipitate was separated off and dried. The yield of WSPSs was 4.1 g. In a hydrolysate PC (system 1; revealing agent 1) revealed traces of galacturonic acid and also galactose, glucose, and mannose in a ratio of 1.4:1:1:51.3 (GLC).

Isolation of the PSs. The residue of the raw material was extracted with a mixture of 0.25% solutions of ammonium oxalate and oxalic acid (1:1) at 70°C (1 × 300 and 1 × 200 ml). The extracts were combined, dialyzed, evaporated, and precipitated with acetone (1:5). The yield of PSs was 3.45 g. In hydrolysates PC (system 1; revealing agent 1) showed the presence of galacturonic acid and neutral monosaccharides: galactose, glucose, xylose, and rhamnose in a ratio of 1.5:25.4:1:1.3 (GLC). The reaction of the PSs with iodine was positive.

Isolation of Hemicelluloses A and B. The residue of the raw material was extracted with 400 ml of a 10% solution of KOH for 2 h with stirring. Then the extract was filtered, the filtrate was neutralized with acetic acid, and the precipitate that had deposited was centrifuged off, washed with acetone, and dried. This gave 1.3 g of hemicellulose A. In hydrolysates of it, PC (system 1; revealing agent 1) showed the presence of galacturonic acid, glucose, arabinose, mannose, and rhamnose. The ratio of the neutral sugars according to GLC results was 25.2:1:1.5:1.6. With iodine, a solution of the HC-A gave a positive reaction, i.e., a blue coloration. The mother solution was evaporated, dialyzed, and precipitated with acetone (1:4). The precipitate was separated off and dried. The yield of hemicellulose B was 2.23 g. In a hydrolysate, PC under the same conditions showed the presence of traces of galacturonic acid, glucose (main spot), mannose, and xylose in a ratio of 42:1.5:1. With iodine, the HC-B gave a positive reaction.

Fractionation of the WSPSs on DEAE-Cellulose. A solution of 2 g of the WSPSs in 100 ml of water was deposited on a column (2.5 × 30 cm) of DEAE-cellulose (acetate form). The neutral polysaccharide was eluted with 500 ml of water, and the acidic polysaccharide with 200 ml of 0.1 N NaOH. The yield of the NPS was 1.2 g, and in a hydrolysate of it PC (system 1; revealing agent 1) revealed mainly mannose together with very small amounts of galactose

and glucose. The yield of the acidic polysaccharide was 0.4 g and in its hydrolysate PC showed the presence of galacturonic acid, galactose, glucose, and mannose.

Gel Filtration of the NPS. A solution of 10 mg of the polysaccharide in 1 ml of water was deposited on a column (1.3 × 50 cm) of Sephadex G-100. Elution was performed with water, the eluates being collected in 3-ml fractions which were analyzed by the phenol/sulfuric acid method [11]. The column was calibrated with dextrans having molecular weights of 80,000 (V_{e_1} 20 ml) and 40,000 (V_{e_2} 23 ml). The NPS had V_{e_3} 18 ml and $V_{e_3'}$ 20 ml.

Fractionation of the NPS. A solution of 1 g of the neutral polysaccharide in 200 ml of water was vigorously stirred while ethanol was added dropwise. Fraction I was obtained on the addition of 260 ml of ethanol, and fraction II on the addition of another 130 ml. The mother solution was evaporated and precipitated with ethanol (1:3), giving fraction III with a yield of 0.35 g. In a hydrolysate of fraction I, PC (system 1; revealing agent 1) showed the presence mainly of mannose, and also trace amounts of glucose, while mannose, galactose, and glucose were found in the hydrolysates of fractions II and III.

Gel Filtration of Ungeromannan-Tr. A solution of 10 mg on fraction I in 1 ml of water was deposited on a column of Sephadex G-100. V_{e_4} was 19.5 ml, which corresponds to a molecular weight of 90,000.

The quantitative determination of O—Ac groups was carried out as described in [2]. Found: O—Ac groups, 4.5%.

Periodate Oxidation and Smith Degradation of Ungeromannan-Tr. The deacetylation of 200 mg of polysaccharide as described in [2] yielded 150 mg of deacylated ungeromannan (DAU). A suspension of 100 mg of DAU in 50 ml of 0.05 M sodium periodate solution was left in the dark at room temperature (+12°C) for 15 days. The oxidation product was reduced with sodium tetrahydroborate and hydrolyzed with 5 ml of 0.5 N H_2SO_4 at 100°C for 6 h. Erythritol and traces of glycerol were found in the hydrolysate by PC (system 1; revealing agents 1 and 2).

Methylation of Ungeromannan-Tr. The polysaccharide (50 mg) was methylated twice by Hakomori's method [6]. This gave 40 mg of completely methylated polysaccharide, the IR spectrum of which contained no absorption band of a hydroxy group. The formolysis and hydrolysis of the permethylate were carried out as described by Bouveng et al. [12], and 2,3,6-tri-O-methyl-D-mannose and trace amounts of 2,3,4,6-tetra-O-methyl-D-mannose and 2,3,6-tri-O-methyl-D-glucose were detected by TLC (systems 2 and 3; revealing agent 1).

Partial Hydrolysis of Ungeromannan-Tr. A solution of 100 mg of DAU in 15 ml of 90% formic acid was brought to a concentration of about 45% and hydrolysis was carried out at 80°C for 4 h. After cooling, the solution was centrifuged and evaporated to dryness, and hydrolysis with 0.5 N H_2SO_4 was carried out at 100°C for 10 min. In the hydrolysate, mannose, mannobiose, and mannotriose, with R_m 0.6, 0.28, and 0.11 were identified with markers.

Degradation of the Ungeromannan-Tr. The polysaccharide (0.5 g) was dissolved in 0.1 N HCl (50 ml) and was hydrolyzed at 85°C for 45 min. The hydrolysate was cooled to room temperature and was precipitated with 150 ml of ethanol. The precipitate was washed with ethanol and was dried over P_2O_5. This gave 0.36 g of a polysaccharide which was used for recording the ^{13}C NMR spectrum.

SUMMARY

1. The group carbohydrate composition of the bulbs of Ungernia trisphaera has been studied. The presence of monosaccharides, water-soluble polysaccharides, pectin substances, and hemicelluloses has been shown. Their quantitative monosaccharide compositions have been established.

2. The homogeneous ungeromannan-Tr, acetylated in the native state, has been isolated by fractionating the neutral polysaccharides. It has been shown on the basis of the results of periodate oxidation, methylation, partial hydrolysis, and IR and ^{13}C NMR spectroscopy that ungeromannan-Tr consists of a linear polymer of D-mannopyranose with β-(1 → 4)-glycosidic bonds.

LITERATURE CITED

1. M. Kh. Malikova, D. A. Rakhimov, and E. S. Kondratenko, Khim. Prir. Soedin., 381 (1983).

2. M. Kh. Malikova, D. A. Rakhimov, and Z. F. Ismailov, Khim. Prir. Soedin., 770 (1983).
3. M. Kh. Malikova, D. A. Rakhimov, and Z. F. Ismailov, Khim. Prir. Soedin., 510 (1981).
4. A. V. Obolenskaya, in: Practical Work in the Chemistry of Wood and Cellulose [in Russian], Moscow (1965), p. 381.
5. S. Hakomori, J. Biochem. (Tokyo), 55, 205 (1964).
6. M. Kh. Malikova, D. A. Rakhimov, and Z. F. Ismailov, Khim. Prir, Soedin., 434 (1981).
7. A. S. Shashkov and O. S. Chizhov, Bioorg. Khim., 2, 437 (1976).
8. T. Usui, T. Mazino, K. Kato, M. Tomoda, and G. Miyasima, Agr. Biol. Chem., 43, No. 4, 863 (1979).
9. I. M. Hais and K. Macek, Paper Chromatography, Academic Press, New York, 3rd edn. (1963).
10. Yu. S. Ovodov, The Gas-Liquid Chromatography of Carbohydrates [in Russian], Vladivostok (1970).
11. M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, Anal. Chem., 28, 350 (1956).
12. H. Bouveng, H. Kiessling, B. Lindberg, and J. E. McKay, Acta Chem. Scand., 16, 615 (1962).

A BIOGLYCAN FROM THE GASTROPOD MOLLUSC Rapana thomasiana

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A bioglycan – rapanan – has been isolated from the mantle of the gastropod mollusc Rapana thomasiana, the main carbohydrate chain of which is constructed of α-(1 → 4)-bound D-glucose residues with a small number of side chains attached by α-(1 → 6)-glucosidic bonds to the main chain of the bioglycan.

In recent years, the urgent attention of research workers has been attracted by bioglycans (polysaccharides and glycoconjugates) from marine organisms, which possess a pronounced immunostimulating action [1].

In the present paper we consider the results of a study of a bioglycan isolated with a yield of 4-5% from the mantle of the gastropod mollusc Rapana thomasiana collected in the Azov–Black Sea basin and in the Sea of Japan.

The bioglycan, which was obtained by the aqueous salt extraction of the mantle of Rapana thomasiana after dialysis and lyophilization, has been called rapanan. In a hydrolysate of it only D-glucose was detected and, therefore, rapanan is a D-glucan. The amount of protein in rapanan does not exceed 1% and it is apparently possible to eliminate the protein completely. According to the results of gel filtration on Sephadex G-100 and Sepharose 6B, rapanan is a high-molecular-weight bioglycan with a molecular weight exceeding 2 MD. The high positive angle of rotation, $[\alpha]_D$ +154° (c 0.1; water), indicates the α configuration of the glycosidic bonds.

The action of α- or β-amylase on rapanan gave a mixture of oligosaccharides and partially degraded glucan. The latter was studied with the aid of paramagnetic spectroscopy and ^{13}C nuclear magnetic resonance. In the PMR spectrum, two broadened signals were observed in the region of the resonance of anomeric protons at δ 5.48 and 5.09 ppm with an integral ratio of 8:1, respectively.

In the ^{13}C NMR spectrum there were likewise two signals in the region of anomeric C atoms at δ 100.6 and 99.3 ppm, in the same integral ratio. The ring C-atoms participating in the formation of the glycosidic bond resonated at 78.6 and 68.2 ppm. These results showed, according to literature information [2, 3], that the glucan under investigation had α-(1 → 4)- and α-(1 → 6)-glucosidic bonds in an approximate ratio of 8:1.

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