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POLYSACCHARIDES OF *Ungernia*.

VII. PARTIAL HYDROLYSIS OF THE MANNAN OF THE BULBS OF *U. vvedenskyi*

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UDC 547.917

4-O-β-D-Manopyranosyl-D-mannose, O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-D-mannose, and O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-D-mannose have been isolated from the products of the partial hydrolysis of ungeromannan-V, obtained from the bulbs of *Ungernia vvedenskyi*, and have been identified. This set of oligosaccharides confirms the regular structure of the carbohydrate chain of ungeromannan-V, which consists of a linear sequence of β-1→4-bound D-mannose residues.

Information on the structure of ungermannan-V obtained on the basis of chromium trioxide and sodium periodate oxidation and methylation has been reported previously [1]. It was established that the monosaccharide residues in it are linked by β-1→4 glycosidic bonds.

For an additional confirmation of the results of methylation, we have studied the structure of ungermannan-V with the aid of ^{13}C NMR spectra and partial hydrolysis. The partial hydrolysis of deacetylated ungermannan-V [1] with formic acid yielded a mixture of mono- and oligosaccharides which was separated by preparative PC. Three chromatographically individual oligosaccharides (I-III) were isolated. Their structures were established from the results of acid hydrolysis, periodate oxidation, methylation, and mass spectrometry.

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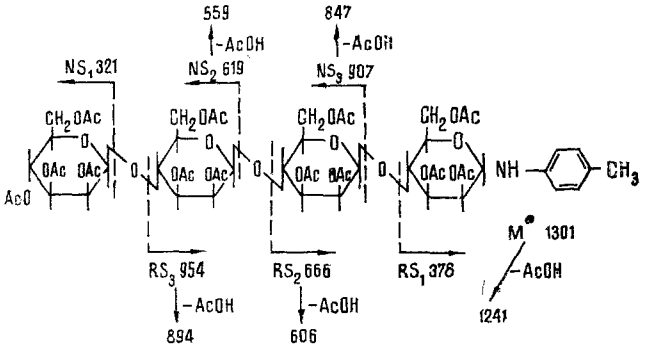
Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 434-436, July-August, 1981. Original article submitted February 9, 1981.

0009-3130/81/1704-0317$07.50

The identification of the products of hydrolysis, oxidation, and methylation was carried out by PC, TLC, and GLC and by comparison with authentic samples. The results obtained are given below:

Oligosaccharide	R_m (PC)	$[\alpha]_D^{22}$ (c 1; water)	Monosaccharide composition	Composition of the oligosaccharide permethylate		DP	Structure
				2,3,4,6-Treta-O-Me-D-Man	2,3,6-Tri-O-Me-D-Man		
I	0,64	- 7.5	Man	1	1	2	4-O-β-*D*-ManpD-Manp
II	0,32	20.0	Man	1	2	3	O-β-*D*-Manp(1→4)-O-β-*D*-Manp(1→4)-*D*-Manp
III	0,11	- 28,5	Man	1	3	4	O-β-*D*-Manp(1→4)-O-β-*D*-Manp(1→4)-O-β-*D*-Manp(1→4)-*D*-Manp

A mass-spectrometric study of the oligosaccharides (II) and (III) was carried out with the completely acetylated N-p-tolylglycosylamines [2]. As has been shown [3], the mass spectra of the derivatives contain peaks corresponding to the molecular ions and also the peaks of two series of ions arising through the cleavage of the bonds between the monosaccharide residues and the corresponding reducing (RS series) or nonreducing (NS series) ends of the oligosaccharide derivative. The origin of the main peaks in the mass spectra of the compounds studied is illustrated by the scheme of the mass-spectrometric fragmentation of the N-p-tolylglycosylamine from mannotetraose.



It was established that the oligosaccharide (II) and (III) had degrees of polymerization of 3 and 4, respectively.

The methylation results were also confirmed by the results of ^{13}C NMR spectroscopy of ungermannan-V. In the region of signals corresponding to the glycosidic C_1 atoms there is a signal at 100.4 ppm corresponding to a β-1→4 bond between mannose residues. A signal at 76.2 ppm corresponds only to C_4 of a β-1→4-bound mannan [4]. The other strongest signals corresponding to mannose residues can be ascribed on the basis of literature information [5, 6] to C_2 (70.5 ppm), C_3 (72.1 ppm), C_5 (75.2 ppm), and C_6 (61.1 ppm). In the ^{13}C NMR spectrum, a signal at 20.7 ppm witnesses the presence of a O—Ac group in some of the mannose residues of ungermannan-V, most probably at C-2 or C-3.

On the basis of the results given in the present paper, to ungermannan-V from the bulbs of *U. vvedenskyi* can be ascribed a regular structure of the carbohydrate chain consisting of a linear sequence of β-1→4-bound D-mannopyranose residues. Such a sequence is also characteristic for the mannans of other plants [7-9]. Ungermannan-V differs from the latter by the degree of polymerization and the number of O-Ac groups.

EXPERIMENTAL

Analytical and preparative PC was carried out on FN 11, 13, and 14 papers in the solvent systems 1) butan-1-ol—pyridine—water (6:4:3) and 2) ethyl acetate—pyridine—water (7:2:1), and thin-layer chromatography (TLC) was carried out on Silufol plates using the systems: 3) methyl ethyl ketone—1% aqueous ammonia (30:4) and 4) benzene-acetone-water (5:5:1). The spots were revealed with: 1) aniline hydrogen phthalate and 2) periodate—$KMnO_4$—benzidine.

The GLC of the samples was performed on a Tsvet 101 instrument with a flame-ionization detector. GLC conditions: stainless steel column (200 × 0.3 cm), 5% of silicone XE-60 on

Chromaton NAW, 0.200 × 0.250; 210°C; carrier gas helium, 60 ml/min. The acetates of the aldononitriles and the acetates of the polyols of the partially methylated sugars were obtained as described by Ovodov [10].

Mass spectra were taken on an MKh-1310 instrument with direct introduction of the sample into the ion source at a temperature of the heater of 210°C and of the ionization chamber of 230°C using an ionizing energy of 50 eV.

The ^{13}C NMR spectra were obtained on a WP-60 spectrometer (Bruker) at 15.08 Hz for a 3% solution of the mannan in D_2O at 90°C. The chemical shifts of the signals were measured relative to DMSO as internal standard.

Partial Hydrolysis of Deacetylated Ungermannan-V (DAU). The DAU (1 g) was dissolved in 80 ml of 90% formic acid, the solution was diluted to a concentration of 45%, and hydrolysis was carried out at 85°C for 3.5 h. Then the solution was cooled, centrifuged, and evaporated to dryness, and the residue was hydrolyzed with 0.5 N H_2SO_4 in the boiling water bath for 10 min. The hydrolysate was neutralized, deionized, chromatographed, and separated preparatively by PC (systems 1 and 2; revelaing agent 1). The R_m values of the oligosaccharides are given above.

Periodate Oxidation and Smith Degradation of the Oligosaccharides (I-III). Each oligosaccharide (10 mg) was oxidized with 10 ml of 0.05 M solution of osdium periodate (+10°C, 48 h). Two drops of ethylene glycol were added and reduction was carried out with 50 mg of sodium tetrahydroborate for 2 h. The reaction mixture was treated with a cation-exchange resin and evaporated, and the residue was hydrolyzed with 0.5 N sulfuric acid at 100°C for 4 h. Glycerol and erythritol were found in the hydrolysate by PC (system 1, revealing agent 2). The hydrolysate was acetylated and analyzed by GLC.

Methylation of Oligosaccharides (I-III). Each oligosaccharide (10 mg) was methylated by Hakomori's method [11]. The permethylate obtained was hydrolyzed with 1 ml of 90% formic acid at 100°C for 1 h, the reaction mixture was evaporated to dryness, and the residue was hydrolyzed with 0.5 N H_2SO_4 on the boiling water bath for 4 h. The hydrolysate was neutralized and deionized, and the TLC (systems 3 and 4; revealing agent 1) showed the presence of 2,3,4,6-tetra-O-methyl-D-mannose (R_f 0.81) and 2,3,6-tri-O-methyl-D-mannose (R_f 0.48). The hydrolysate was reduced and acetylated and was then analyzed by GLC.

Preparation of Acetylated N-p-Tolylglycosylamines. The oligosaccharides (II) and (III) were converted into derivatives by the method of Usov and Barbakadze [2].

CONCLUSION

The structure of ungermannan-V, isolated from the bulbs of *U. vvedenskyi* has been studied with the aid of ^{13}C NMR spectrsocopy and partial hydrolysis. The results obtained show a regular structure of the carbohydrate chain of ungermannan-V with a linear sequence of β-1→4-bound D-mannopyranose residues.

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