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COMPARATIVE STRUCTURAL STUDY OF THE LIPOPOLYSACCHARIDES OF Y. enterocolitica SEROVARS O:7.8 AND O:19.8

S. V. Tomshich, R. P. Gorshkova, and Yu. S. Ovodov

UDC 579.842.23+577.114.5+543.544.45+51

The lipopolysaccharides of Yersinia enterocolitica, serovars O:7.8 (strain 106) and O:19.8 (strain 842), isolated from the microbial mass by phenol-water extraction, contained residues of L-fucose, 6-deoxy-D-gulose, D-mannose, D-galactose, D-glucose, D- and L-glycero-D-mannoheptoses, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, and 2-keto-3-deoxyoctonic acid (KDO). The polysaccharides obtained by mild acid hydrolysis of the lipopolysaccharides followed by gel filtration on Sephadex G-50 were a mixture of the O-specific polysaccharide and the core, which could not be separated even by repeated rechromatography because of the comparability of their molecular masses. On the basis of the results of monosaccharide analysis, methylation, Smith degradation, and partial hydrolysis, a structure has been suggested for the repeating unit of the O-specific polysaccharides of the lipopolysaccharides of Y. enterocolitica of the serovars studied.

We have previously suggested a structure for the repeating unit of the O-specific polysaccharide of the Y. enterocolitica, serovar O:8 (strain 161) [1]. The present work is a continuation of the structural study of the lipopolysaccharides (LPS) of the microorganism causing yersiniosis, Y. enterocolitica.

The LPS were isolated from a dry acetone powder of the microbial mass of Y. enterocolitica of serovars O:7.8 (strain 106) and O:19.8 (strain 842) by phenol-water extraction according to Westphal [2]. To determine their monosaccharide compositions, the LPS were subjected to acid hydrolysis, and the monosaccharides were identified with the aid of paper chromatography (PC), GLC, and chromato-mass spectrometry (GLC-MS) in the form of polyol acetates [3]. The LPS investigated had the same monosaccharide composition and contained residues of L-fucose, 6-deoxy-D-gulose, D-mannose, D-galactose, D-glucose, D- and L-glycero-D-manno-heptoses, D-glucosamine, D-galactosamine, and 2-keto-3-deoxyoctonic acid (KDO) (Table 1).

The polysaccharides (PS) obtained by mild acetic-acid hydrolysis of the LPS followed by gel filtration on Sephadex G-50 and G-25 consisted of the O-specific polysaccharide attached to the core, as in the case of the polysaccharide of serovar O:8 [1], and contained all the monosaccharide residue of the initial LPS with the exception of the KDO (see Table 1). This can probably be explained by the assumption that the O-specific chains and core have comparable molecular masses and are eluted together on gel filtration. The lability of the 6-deoxy-D-glucose bond must be mentioned, since even under the conditions of mild acid hydrolysis a considerable amount of it was split out and was isolated in the pure form. In view of this, the PS were distinguished by a lower content of this sugar.

To establish the link between the monosaccharide residue, the LPS of Y. enterocolitica, serovar O:7.8 and O:19.8 and of the PS obtained from them were methylated by Hakomori's method [4] followed by methanolysis (or hydrolysis). The mixture of partically methylated monosaccharides so obtained was analyzed in the form of methyl glycosides and polyol acetates with the aid of GLC [5] and of GLC-MS [3, 6]. It is obvious from the results on the methyla-

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TABLE 1. Monosaccharide Composition of the Lipopolysaccharides (LPS) and Polysaccharides of Yersinia enterocolitica, serovars O:7.8 and O:19.8

Serovar	Strain		L-Fuc	6d-D-Glc	Man	Gal	Glc	Hep (D—D+ —L—D)	HexN (GalN+GlcN)
O:7,8	106	LPS	1	1,7	1	1	1	1,2 (1:1,5)	+
		PS	1	1	1	1	1,5	2,5 (1:3)	+
		OPS-1	Tr.	—	2,3	1,9	—	1 (*L—D*)	+ (1:1)
		OPS-2	—	—	2,2	2	—	—	+ (1:1)
O:19,8	842	LPS	1	1,5	1	1	0,8	0,8 (1:1,5)	+
		PS	1	1	1	1	1	1,5 (1:3)	+
		OPS-1	—	—	1,2	1	—	0,5 (*L—D*)	+ (1:1)

Note: OPS-1 and OPS-2 are polysaccharides obtained after one and two Smith degradations, respectively.

tion of the LPS and PS (Table 2) that the L-fucose and 6-deoxy-D-gluose residues were present at the nonreducing ends of the polysaccharide chain while the hexose (mannose, glucose, and galactose) residues were present both in the main chain and in branches. The amino sugars were present only as components of the main polysaccharide chain. All the monosaccharide residues existed in the pyranose form. The methyl ethers of both heptoses of the core were present in great variety and amount.

The polysaccharides of the Y. enterocolitica serovars were subjected to Smith degradation [7]. The products of the first oxidation of the PS of serovars O:7.8 and O:19.8 contained mannose, galactose, L-glycero-D-mannoheptose, glucosamine, and galactosamine residues (Table 1). The practically complete oxidation of the 6-deoxyhexoses confirmed the methylation results indicating that they occupied terminal positions. The glucose and D-glycero-D-mannoheptose residues were oxidized completely. The polysaccharides modified after the first Smith degradation (OPS-1) were methylated and subjected to methanolysis, and the products were investigated with the aid of GLS-MS (Table 2). The identification of 2,4,6-tri-O-methyl-D-galactose (in place of the 4,6-di-O-methyl-D-galactose in the case of the initial polysaccharides) showed that the galactose residue was included in the main polysaccharide chain by a 1,3-bond, and the residue of one of the oxidation 6-deoxyhexoses was attached to the main chain by a 1,2-bond. The appearance of 2,3,4,6-tetra-O-methyl-D-mannose and 3,4,6-tri-O-methyl-D-mannose formed from terminal and interior units, respectively, (in place of the 2,6-di-O-methylmannose and 6-O-methylmannose in the case of the initial PS) can be explained by the assumption that the mannose residue was included in the main chain by a 1,2-bond and the side chains that were oxidized by the periodate were attached by 1,3- and 1,4-bonds. Because of the short length of the O-specific chains in the LPS of the serovars studied (2-3 repeating units), the above-mentioned methyl ethers of mannose were formed in comparable amounts in the initial and in the oxidized polysaccharides. The presence of 2,4,6-tri-O-methyl-D-mannose in methanolysates of OPS-1 was due to the partial oxidation of the 1,3-bound heptose of the core to mannose.

The polysaccharide of serovar O:7.8 (strain 106), after the first oxidation, was subjected to a repeat Smith degradation. The polysaccharide so obtained (OPS-2) contained the monosaccharide residues of the O-specific chain – galactose and galactosamine – and of the core – glucosamine, and also the mannose formed in the oxidation of the L-glycero-D-mannoheptose. 2,3,4,6-Tetra-O-methylgalactose, 2,4,6-tri-O-methylmannose, 4,6-di-O-methylglucosamine, and 4,6-di-O-methylgalactosamine were identified in a methanolysate of this methylated OPS-2.

To establish the sequence of monosaccharide residues in the polysaccharide chain, we carried out the partial hydrolysis of the polysaccharide of Y. enterocolitica, serovar O:19.8 (strain 842) followed by preparative paper chromatography. Table 3 gives the monosaccharide compositions of some of the oligosaccharide fractions isolated, which were determined with the aid of GLC. As can be seen, all the oligosaccharide fractions had practically the same monosaccharide composition, the main components being mannose, galactose, and an amino sugar

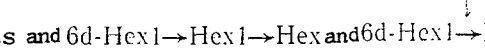
TABLE 2. Results of the Methylation of the LPS and Their Derivatives from Y. enterocolitica, serovars 0:7.8 and 0:19.8

Methylated monosaccharide	Serovar 0:7.8 strain				Serovar 0:19.8 (strain 842)		
	LPS	PS	OPS-1	OPS-2	LPS	PS	OPS-1
2, 3, 4-OMe_3-6d-*D*-Gul	+	+	—	—	+	+	—
2, 3, 4-OMe_3-*L*-Fuc	+	+	—	—	+	+	—
2, 3, 4, 6-OMe_4-*D*-Glc	+	+	—	—	+	+	—
(*D*-Man)			+	—			+
3, 4, 6-OMe_3-*D*-Man	—	—	+	—	—	—	+
2, 4, 6-OMe_3-*D*-Man	—	—	+	+	—	—	+
2, 3, 6-OMe_3-*D*-Glc	+	+	—	—	+	+	—
(*D*-Man)	—	+	—	—	—	+	—
3, 6-OMe_2-*D*-Man	—	+	—	—	—	+	—
2, 6-OMe_2-*D*-Man	+	+	—	—	+	+	—
6-OMe-*D*-Man	+	+	—	—	+	+	—
2, 3, 4, 6-OMe_4-*D*-Gal	—	—	Tr.	+	—	—	Tr.
2, 4, 6-OMe_3-*D*-Gal	—	—	+	—	—	—	+
4, 6-OMe_2-*D*-Gal	+	+	—	—	+	+	—
2, 3, 4, 6, 7-OMe_5-*D*, *D*-Hep	+	+	—	—	+	+	—
2, 3, 4, 6, 7-OMe_5-*L*, *D*-Hep	+	+	—	—	+	+	—
2, 3, 4, 6-OMe_4-Hep	+	+	—	—	+	+	—
2, 4, 6, 7-OMe_4-*L*, *D*-Hep	—	—	+	—	—	—	+
2, 6, 7-OMe_3-Hep	+	+	—	—	+	+	—
2, 4, 6-OMe_3-Hep	+	+	—	—	+	+	—
6, 7-OMe_2-Hep	+	+	—	—	+	+	—
4, 6-OMe_2-*D*-GlcN	+	+	+	+	+	+	+
4, 6-OMe_2-*D*-GalN	+	+	+	+	+	+	+

Note: OPS-1 and OPS-2 are the polysaccharides obtained after one and two Smith degradations, respectively.

(galactosamine), the others being present in trace amounts. The assumption that the oligosaccharide fractions (individual, according to the results of PC) consisted of mixtures of several oligosaccharides was confirmed by their analysis with the aid of GLC-MS [8-10] in the form of methyl ethers (Table 3).

In the oligosaccharides of fraction B the disaccharide Hex1 → 3Hex was identified from its mass spectrum (m/z (%): 279 (abJ, 4), 219 (aA_1, 17), 187 (aA_2, 21), 159 (19), 155 (aA_3, 8), 149 (11), 145 (13), 127 (17), 125 (16), 101 (64), 88 (100), 75 (43), 71 (90), the relative intensities of the peaks of the ions being given in parentheses and the designation of the ions by the symbols A-J agreeing with that proposed previously [9]; the presence of ions with m/z 159 and 145 showed a 1,3-bond between the monosaccharide residues and the disaccharide [10]); and so was the triaccharide Hex1 → Hex1 → HexN [m/z (%): 668 (M−31, 0.5), 622 (0.5), 610 (0.6), 549 (0.5), 524 (abcJ, 0.8), 464 (bcA_1, 1), 432 (bcA_2, 3), 423 (baA_1, 6), 391 (baA_2, 3), 359 (baA_3, 2.5), 260 (cA_1, 100), 228 (cA_2, 25), 219 (aA_1, 100),, 205 (13), 187 (aA_2, 95), 155 (aA_3, 18), 101 (85), 88 (80), 85 (85), 83 (80)]. The oligosaccharide fraction B included residues of mannose, galactose, and galactosamine. In a methanolysate of its fully methylated derivative, 2,3,4,6-tetra-O-methylmannose, 2,4,6-tri-O-methylgalactose, and 4,6-di-O-methylgalactosamine were identified. On this basis, the oligosaccharides mentioned above had the following structures: Man1 → 3Gal, Man1 → 3Gal1 → 3GalN. This shows that in the main polysaccharide chain the mannose residue was attached by a 1,3-bond to a galactose residue, which in its turn was linked to C-3 of a galactosamine residue. In the oligosaccharide fraction C



a mixture of the trisaccharides and 6d-Hex1→Hex1→Hex and 6d-Hex1→Hex was identified with the aid of mass spectrometry.

On the basis of what has been stated above it may be assumed that the main chain of the O-specific polysaccharide of Y. enterocolitica, serovars 0:19.8 and 0:7.8 is constructed of D-mannose, D-galactose, and galactosamine residues. 6-Deoxyhexoses are attached in the form of side-chains to the main chain of the O-specific polysaccharide, while L-fucose glycosylates the galactose residue in the second position (appearance of 2,4,6-tri-O-methylgalactose in a methanolysate of the methylated OPS-1 after the elimination of fucose). Two 6-deoxy-D-gulose residues are attached to the mannose residues in positions 3 and 4, as was shown by the larger amount of this monosaccharide in the initial LPS, its double integral intensity in the ^{13}C NMR spectrum of the polysaccharide as compared with fucose, and the appearance of 2,3,4,6-tetra-O-methylmannose and 3,4,6-tri-O-methylmannose in the methanolysate of methylated OPS-1 (after the oxidation of the 6-deoxygulose). In addition, one may note the appearance in the

TABLE 3. Oligosaccharide Fractions Obtained from the Polysaccharide of Y. enterocolitica Serovar O:19.8

Oligosaccharide fraction	R_{gal}	$[\alpha]_{578}^{20}$	Monosaccharide composition							Oligosaccharides identified from their mass spectra	Partially methylated monosaccharides composition
			Fuc	6d-Gal	Man	Gal	Glc	Hep	HexN		
A	0,14	5	Tr.	Tr.	1	1	0,3	0,5	+ (GalN)	1. Hex1→Hex	2, 3, 4, 6-OMe_4-Man (Gal)
										2. Hep1→Hep	2, 3, 4, 6, 7-OMe_5-Hep
										3. Hex1→Hex1→HexN	2, 4, 6-OMe_3-Gal
											4, 6-OMe_2-HexN
B	0,21	22	Tr.	Tr.	1	1	Tr.	—	+ (GalN)	1. Hex1→3Hex	2, 3, 4, 6-OMe_4-Man
										2. Hex1→Hex1→HexN	2, 4, 6-OMe_3-Gal
											4, 6-OMe_2-HexN
C	0,31	0	0,5	Tr.	1	1	0,3	1	+ (GalN)	1. Hep1→Hep	2, 3, 4, 6-OMe_4-Man (Gal)
										2. 6d-Hex1→Hex1→Hex	2, 3, 4, 6, 7-OMe_5-Hep
										Hex1→Hex	2, 3, 4-OMe_3-6d-Hex
										↑ 6 6d-Hex	2, 4, 6-OMe_3-Gal
										3. Hex1→Hex1→HexN	4, 6-OMe_2-HexN
D	0,1	4	Tr.	Tr.	1	1	Tr.	—	+ (GalN)	1. Hex1→3Hex	2, 3, 4, 6-OMe_4-Man (Gal)
										2. Hex1→HexN	2, 4, 6-OMe_3-Gal
											4, 6-OMe_2-HexN

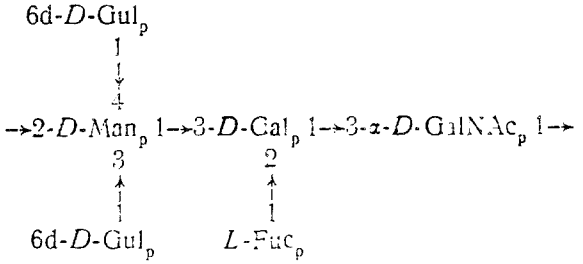
*The R_{Gal} values were determined on FN-15 paper in the butan-1-ol—pyridine—water (6:4:3) system.

methylated PS (i.e., after the partial splitting out of 6-deoxygulose on the weak acid hydro hydrolysis of the LPS) of 2,3,6-tri-O-methylmannose and 3,6-di-O-methylmannose. The mannose and galactose residues are included in the main chain by 1,2- and 1,3-bonds, respectively.

The ^{13}C NMR spectra of the initial polysaccharides and those obtained after Smith degradation (OPS-1 and OPS-2) could not be interpreted completely for either of the serovars because of their complexity. Thus, 8-10 signals were observed merely in the region of resonance of the anomeric carbon atoms in the spectra of the initial PS. Signals with chemical shifts of 15.7 and 16 ppm related to the carbon atoms of the methyl groups of 6-deoxyhexose (6-deoxy-D-gulose and -L-fucose, respectively), and these had practically disappeared already in the spectra of the OPS-1, i.e., after the first oxidation. The signals of two acetyl groups at 22.7 and 23.6 ppm and also at 174 and 174.4 ppm and of two carbon atoms linked to nitrogen (49.6 and 55 ppm) showed that they included N-acetylglucosamine and N-acetylgalactosamine residues; at the same time, these signals were retained in the spectra of OPS-1 and OPS-2 and, consequently, the above-mentioned residues were glycosylated in the third position. The values of the chemical shifts of the secondary carbon atoms showed that the N-acetylglucosamine had the β-configuration and the N-acetylgalactosamine the α-configuration [11].

From the results obtained it is impossible to show a difference in the structure of the O-specific polysaccharides of Y. enterocolitica, serovars O:7.8 (strain 106) and O:19.8 (strain 842). There are probably some differences in them in the configurations of the glycosidic bonds which we were unable to establish. We must also mention the high content of OAc groups in the LPS and PS of serovar O:19.8 (2.2 and 1.04%) as compared with serovar O:7.8 (0.75 and 0.75%).

Summarizing all the results obtained and taking what has been said above into account, it is possible to propose the following structure of the repeating unit of the O-specific polysaccharide of Y. enterocolitica, serovars O:19.8 and O:7.8:



The presence of the common factor 8 in the LPS of Y. enterocolitica of serovars O:19.8 and O:7.8 and the serovar O:8 studied previously [1] was confirmed by the existence of cross precipitation reactions between the LPS of all the serovars and antisera to them.

EXPERIMENTAL

Descending chromatography on Filtrak FN-12 and FN-15 papers was conducted in the butan-1-ol–pyridine–water (6:4:3, by volume) system, the monosaccharides being detected with alkaline silver solution and the amino sugars with a 2% solution of ninhydrin in acetone.

Gel filtration was carried out on columns of Sephadexes G-50 (2 × 60 cm), G-25 (2 × 65 cm) and G-15 (2 × 65 cm) in pyridine-acetate buffer [pyridine–acetic acid–water (10:4:986, by volume)], and on TOYOPEARL HW-50F (2 × 70 cm) in water, the fractions being analyzed by the phenol/sulfuric acid method. GLC was performed on a Pye-Unicam-104 chromatograph (United Kingdom) with a flame-ionization detector in glass column (0.4 × 150 cm) containing: 3% of QF-1 on Gas-Chrom Q (100-120 mesh) – column A; 3% of OV-225 on Gas-Chrom Q (100-120 mesh) – column B; and 5% of SE-30 on Chromaton (100-120 mesh) – column C; the carrier gas was argon at a rate of flow of 60 ml/min. Acetate of the polyol derivatives of the monosaccharides were analyzed on columns A and B in the temperature interval of 175-225°C (5°C/min), and the acetates of methyl glycosides and of partially methylated monosaccharides on column A at 110-225°C (5°C/min), and fully methylated oligosaccharide fractions on column C in the temperature interval of 200-300°C (5°C min), GLC-MS was carried out on a LKB-9000s instrument using columns with the same phases.

^{13}C NMR spectra of the polysaccharides were recorded on a Bruker-Physik WM-250 instrument in D_2O at 60°C. Methanol was used as internal standard (49.6 ppm). The chemical shifts

were recalculated relative to tetramethylsilane. Optical rotations were measured on a Perkin-Elmer, model 141, polarimeter in water.

The microorganisms of Y. enterocolitica, serovars O:7.8 (strain 106) and O:19.8 (strain 842) were obtained from the Centre International des Yersinia (Paris, Prof. H. H. Mollaret). The cells were grown on a synthetic medium. The LPS were isolated by extraction with 45% aqueous phenol and were purified by three ultracentrifugations at 105,000×g [2]. The LPS were lyophilized, the yield amounting to 1-2% of the weight of the dry cells.

The complete acid hydrolysis of the LPS (10 mg) and the PS (5 mg) was carried out with 0.5 N trifluoroacetic acid, 2 N HCl, at 100°C for 3.5 h. The solutions were evaporated several times with methanol. The monosaccharides were identified by PC and GLC in the form of polyol acetates.

Preparation of the Polysaccharides. The LPS of serovars O:19.8 (500 mg) and O:7.8 (500 mg) were each hydrolyzed with 1% acetic acid (50 ml) on the boiling water bath for 1.5 h. The lipid A that deposited as a precipitate was separated off by centrifugation (15,000 rpm, 30 min). The supernatant was lyophilized and the product was then separated by gel filtration on Sephadex G-50. A polysaccharide fraction and a mixture containing mono- and oligosaccharides and KDO were isolated. The polysaccharide fraction was rechromatographed on Sephadex G-25, giving the polysaccharides (PS) of serovars O:7.8 (200 mg), $[\alpha]_{578}^{20}$ + 34° and O:19.8 (175 mg), $[\alpha]_{578}^{20}$ + 37° containing the O-specific polysaccharide and the core, which were used for subsequent structural investigations.

Analysis by the Methylation Method. The LPS and their fragments (5-10 mg) were first dried over P_2O_5 at 60°C and were then dissolved in dimethyl sulfoxide (0.5-1 ml) and were methylated with methyl iodide (0.5-1 ml) in the presence of the methylsulfinyl carbanion by the standard procedure [4]. The fully methylated compounds (half) were heated in methanolysis mixture (1 N HCl in methanol, 100°C, 3 h) and evaporated several times with methanol, the residue was acetylated with acetic anhydride in pyridine (1:1, 0.4 ml, 100°C, 30 min), and the product was investigated by GLC and GLC-MS. The second half of the methylated compound was subjected to formolysis (0.5 ml, 90% HCOOH, 100°C, 2 h) and the product was evaporated to dryness and was then hydrolyzed with 0.13 M sulfuric acid (0.5 ml, 100°C, 12 h). The products were neutralized with Dowex-1 (HCO_3^- form) and the mixture of methylated sugars was reduced with sodium tetrahydroborate, acetylated, and investigated by GLC and GLC-MS. The results are given in Table 2.

Smith Degradation. The polysaccharides of serovars O:19.8 (80 mg) and O:7.8 (160 mg) were treated with a 0.1 M solution of sodium metaperiodate in water (8 and 15 ml) at 20°C for 67 h in the dark, and then, with cooling, sodium tetrahydroborate (200 mg) was added to the solution in portions, and, after 20 h, the mixture was acidified with acetic acid to pH 5 and was chromatographed on a column of Sephadex G-15 (2.8 × 81 cm) in pyridine-acetate buffer. The polyalcohol obtained was kept in 0.5 N HCl (10 and 15 ml) at room temperature for 70 h. When this solution was subjected to gel filtration on Sephadex G-15, the modified polysaccharides OPS-1 of serovars O:19.8 (22 mg $[\alpha]_{578}^{20}$ + 64°) and O:7.8 (65 mg $[\alpha]_{578}^{20}$ + 69°) were isolated. The latter were subjected to the same treatment again and the twice-oxidized polysaccharide OPS-2 (25 mg, $[\alpha]_{578}^{20}$ + 70°) was obtained.

Partial Hydrolysis. The polysaccharide of serovar O:19.8 (85 mg) was hydrolyzed with 0.5 N trifluoroacetic acid (TFA, 3 ml) on the boiling water bath in a flask with a reflux condenser for 20 min. The TFA was eliminated by evaporation with compressed air. The syrup was dissolved in a small volume of water and was precipitated with a fivefold amount of ethanol. The precipitate was separated off by centrifugation and was dissolved in 0.5 N TFA (2 ml). The initial operation was repeated three times. All the ethanolic solutions were combined and evaporated. The oligosaccharides were isolated with the aid of preparative PC. As a result, four main oligosaccharide fractions were isolated: A, B, C, and D (4, 3, 4, and 2 mg), which were individual according to the results of PC, and their monosaccharide compositions were established after acid hydrolysis (0.5 N TFA, 100°C, 3 h) by GLC. 6-D-Deoxy-D-gulose (12 mg, $[\alpha]_{578}^{20}$ −30°) was isolated in parallel.

SUMMARY

The lipopolysaccharides have been isolated from the microbial mass of Yersinis enterocolitica, serovars O:7.8 (strain 106) and O:19.8 (strain 842). The polysaccharides were obtained by mild acid hydrolysis followed by gel filtration on Sephadexes, and their qualitative and quantitative monosaccharide compositions were established.

On the basis of the results of methylation, Smith degradation, partial hydrolysis, GLC-MS, and ^{13}C NMR spectroscopy a partial structure has been suggested for the repeating unit of the O-specific polysaccharide of the lipopolysaccharides of the serovars investigated.

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CHEMICAL INVESTIGATION OF BIOMASS OF A CULTURE OF GINSENG CELLS

III. POLYSACCHARIDES OF A CALLUS CULTURE OF GINSENG

T. F. Solov'eva, V. A. Khomenko,
N. I. Uvarova, N. A. Konstantinova,
V. S. Faustov, and G. B. Elyakov

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A water-soluble polysaccharide fraction has been isolated from a callus culture of ginseng with a yield of 16-20%. It has been shown that it included starch a and acidic polysaccharides — arabinogalactans and a xyloglucan.

Chemical investigations of the polysaccharides of ginseng that began in the 1960s [1, 2] have been taken up again after a long interval [3, 4]. The renewal of interest in this subject is apparently due in part to advances in the field of ginseng tissue culture which permit the growth of the cell mass of ginseng by an industrial method.

We have isolated a polysaccharide fraction from a culture of ginseng tissue with the aim of the further study of its biological properties. The water-soluble polysaccharides of the cell were isolated by successive extraction with water, ammonium oxalate, and sodium carbonate. The yields and characteristics of the high-molecular-mass fractions obtained are given below (%):

Solvent	Yield	Monosaccharides	Uronic acids	Protein	Ash
Water	16,8	43,4	10,7	2,5	3,2
Oxalate	5,9	31,8	18,9	1,7	2,9
Sodium carbonate	4,9	24,2	8,2	59,5	5,1

Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Branch, USSR Academy of Sciences, Vladivostok, All-Union Scientific Research Institute of Biochemical Machinery Design, USSR Ministry of the Medical Industry, Moscow.

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