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STRUCTURAL INVESTIGATION OF THE LIPOPOLYSACCHARIDE OF *Yersinia enterocolitica* SEROVAR 0:8

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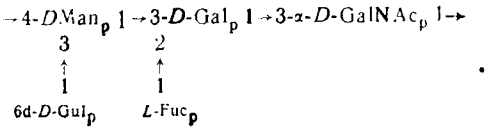
UDC 579.842.23+577.114.5+543.544.45+51

The lipopolysaccharide of *Yersinia enterocolitica* serovar 0:8 (strain 161) isolated from the microbial mass by aqueous–phenol extraction contains 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 polysaccharide was obtained by mild acid hydrolysis of the lipopolysaccharide followed by gel filtration on Sephadex G-50. On the basis of the results of monosaccharide analysis, methylation, Smith degradation, and partial hydrolysis the following structure is suggested for the repeating unit of the O-specific polysaccharide of the LPS of *Yersinia enterocolitica*, serovar 0:8:



In recent years, the microorganism *Yersinia enterocolitica* has been isolated more and more frequently from patients with the symptoms of the intestinal disease called yesriniosis, and also from animals, birds, and the environment. The bulk of publications [1-3] on infections caused by *Y. enterocolitica* relates to its biochemical and serological characterization; the O-anigens of this microorganism have been little-studied structurally [4-6].

The lipopolysaccharides (LPSs) were isolated from a dry acetone powder of the microbial mass of *Y. enterocolitica*, serovar 0:8 (strain 161), by aqueous phenol extraction according to Westphal [7]. The monosaccharide composition of the LPSs was determined (after preliminary hydrolysis with 0.5 N CF_3COOH or 2 N HCl) with the aid of paper chromatography, gas–liquid chromatography (GLC), and chromato-mass spectrometry (GLC-MS) of the corresponding polyol acetates [8]. The following monosaccharides were identified: L-fucose, 6-deoxy-D-gulose, D-mannose, D-galactose, D-glucose, D- and L-glycero-D-mannoheptoses, D-glucosamine, D-galactosamine, and 2-keto-3-deoxyoctonic acid (KDO). The ratio of the monosaccharides in the LPS determined by the GLC of the corresponding polyol acetates was as follows: L-Fuc:6d-D-Gul:D-Man: D-Gal:D-Glc:Hep_{1+2} ∿ 1:1.5:1:1:2:1:3, respectively, the D- and L-glycero-D-mannoheptose residues being in a ratio of 1:3.

To isolate the O-specific polysaccharide, the LPSs were subjected to weak acid hydrolysis, and the lipid A that then precipitated was separated off by centrifugation. In gel filtration on Sephadex G-50, the polysaccharide moiety issued mainly as a single peak (without considering a glucan, amounting to 3-5% of the main fraction). Analysis with the aid of GLC of the monosaccharide composition of the polysaccharide isolated showed that it contained amino sugars (D-GlcN and D-GalN) and all the monosaccharides of the initial LPSs with the exception

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of the KDO in the following ratio: L-Fuc:6d-D-Gul;D-Gal:D-Man:D-Glc:Hep_{1+2} as 1:1:1: 1.5:3, respectively. In spite of the repeated chromatography on Sephadexes G-50 and G-25 and on Toyopearl HW-50, it was impossible to isolate the O-specific polysaccharide free from the core oligosaccharide. It is likely that the O-specific polysaccharide and the core have comparable molecular weights and are eluted together in gel filtration, which greatly complicated all the subsequent structural investigations performed with this combined preparation (having $[\alpha]_{589}^{20}$ + 47°).

To establish the bond between the monosaccharide residues of the LPSs of *Y. enterocolitica*, strain 161, the polysaccharide (PS) obtained from them was methylated by Hakomori's method [9]. The mixture of partially methylated monosaccharides obtained on the hydrolysis of the methylated LPSs and PS was analyzed in the form of the acetates of the corresponding methyl glycosides and polyol acetates with the aid of GLC [10] and of GLC-MS [8, 11]. As can be seen from the results on the methylation of the LPSs and the PS (Table 1), the L-fucose and 6-deoxy-D-glucose residues were present only in the side chains, while the hexose (D-Man, DOGlc, D-Gal) residues were present both in the main chain and in the branches. Amino sugars were present only in the main polysaccharide chain. The great diversity and the amount of methyl ethers of the two heptoses confirmed that there was a considerable amount of core present both in the LPSs and in the polysaccharide. All the monosaccharide residues were present in the pyranose form.

The polysaccharide of *Y. enterocolitica* serovar 0:8 (strain 161) was subjected to Smith degradation [12]. The oxidation product obtained (OPS-I) contained residues of D-mannose, D-galactose, L-glycero-D-mannoheptoses, D-glucosamine, and D-galactosamine, the amount of mannose being twice as great as that of galactose (because of the partial oxidation of the heptoses of the core to mannose, as in the case of the periodate oxidation of the heptoses of the core of *Y. pseudotuberculosis* [13]. Table 1 gives the results of the methylation of this modified polysaccharide. The appearance of 2,4,6-tri-O-methyl-D-Gal in its methanolysate (in place of the 4,6-di-O-methyl-D-Gal in the initial polysaccharide) indicates that the galactose residue in the main polysaccharide chain is bound in position 3, and a substituent, i.e., a side chain (one of the 6-deoxyhexoses that underwent oxidation) is attached in position 2. In the main chain, the mannose residue is bound with C-4, and the side chain is attached in position 3 (2,6-di-O-methyl-D-Man and 2,3,6-tri-O-methyl-D-Man, respectively, before and after oxidation). The appearance of 2,4,6-tri-O-methyl-D-Man is due to the partial oxidation of a 1→3-bound heptose of the core to mannose.

The polysaccharide after the first oxidation was subjected to a second Smith degradation. The degradation products so obtained (OPS-II) contained residues of D-mannose, D-galactose (∿2:1), D-glucosamine, and D-galactosamine, together with traces of L-glycero-D-mannoheptose. 2,3,4,6-Tetra-O-methyl-D-Gal, 2,4,6,tri-O-methyl-Man (the main component), 4,6-di-O-methyl-D-GlcN and -GalN, and traces of 2,4,6,7-tetra-O-methyl-Hep were identified in a methanolysate of this methylated oligosaccharide.

To established the sequence of monosaccharide residues in the O-specific chain, we attempted to obtain oligosaccharides by various methods (partial hydrolysis with 0.25 N H_2SO_4 and with 0.5 N CF_3COOH, and solvolysis of the LPSs) followed by paper chromatography. In all cases a complete set of oligosaccharides was observed without the predominance of any particular one in the mixture, i.e., it is likely that no specific cleavage of the polysaccharide chain took place. Table 2 gives the monosaccharide compositions of some of the oligosaccharide fractions isolated (individual according to paper chromatography), which were determined in the form of the corresponding polyol acetates by GLC. The fact that the oligosaccharide fraction isolated contained almost all the monosaccharides of the initial LPSs indicated that they consisted of a mixture of several oligosaccharides. This hypothesis was confirmed in an analysis of the methylated oligosaccharide fractions with the aid of chromato-mass spectrometry [14-16], i.e., in actual fact each fraction was a mixture of several (3-6) oligosaccharides with different compositions and structures (Table 2). The oligosaccharide fraction A was probably formed from an accompanying glucan, since it contained mainly the disaccharide Hexl→4Hex, and on hydrolysis gave mainly glucose. The mass spectrum of this disaccharide (relative intensities are given in parentheses) was: m/z 305 (7), 279 (11)—abJ, 219 (40)—aA_1, 187 (81)—aA_2, 161 (8), 155 (23)—aA_3, 145 (16), 131 (13), 129 (8), 127 (21), 101 (100), 88 (130), the ions being denoted by the symbols A—J in the manner proposed previously [15]. The presence in the mass spectrum of ions with m/z 305 and 161 showed a 1→4 bond in the disaccharide [14]. In oligosaccharide fraction D the main component according to the mass spectrum was a disaccharide Hexl→3Hex (279 (7)—abJ, 219 (28)—aA_1, 187 (40)—aA_2, 175 (7), 159 (28) 155 (15)—aA_3, 145 (28), 143 (10), 127 (32), 111 (37), 101 (81), 95 (30), 88 (77),

TABLE 1. Partially Methylated Monosaccharides from Hydrolysates of the Methylated Lipopolysaccharides (LPSs) and Methylated Polysaccharide (PS) and Fragments Obtained from Them (OPS-I and OPS-II)

Partially methylated monosaccharides	LPSs	PS	OPS-I	OPS-II
2,3,4-OMe_3-*L*-Fuc	+	+	—	—
2,3,4-OMe_3-6d-*D*-Gul	+	+	—	—
2,3,4,6-OMe_4-*D*-Glc (-*D*-Man)	+++	++	+	—
2,3,4,6-OMe_4-*D*-Gal	Tr.	Tr.	—	+
2,3,4,6,7-OMe_5-*D*, D-Hep	+	+	—	—
2,3,4,6,7-OMe_5-*L*, D-Hep	+	+	—	—
2,3,6-OMe_3-*D*-Glc	+	—	—	—
2,4,6-OMe_3-*D*-Man	—	—	++	+++
2,3,6-OMe_3-*D*-Man	Tr.	+	+	—
2,4,6-OMe_3-*D*-Gal	—	—	++	Tr.
2,3,4,6-OMe_4-Hep	++	++	—	—
2,6-OMe_2-*D*-Man	+++	++	—	—
4,6-OMe_2-*D*-Gal	+++	+++	—	—
2,4,6,7-OMe_4-Hep	—	—	+++	Tr.
2,6,7-OMe_3-Hep	++++	++++	—	—
2,4,6-OMe_3-Hep	++	++	—	—
2,6-OMe_2-Hep	+	+	—	—
4,6-OMe_2-*D*-GlcN	++	++	+	+
4,6-OMe_2-*D*-GalN	++	++	+	+

Note. OPS-I — polysaccharide obtained after the first Smith degradation of the PS; OPS-II — doubly oxidized PS.

81 (50), 75 (58), 71 (100)), the ion with m/z 159 showing the 1→3 bond between the monosaccharide residues in the disaccharide. Trisaccharides 6d-Hex1→Hex1→Hex and Hex1→Hex1→HexN were present in minor amounts, and it was established that the reducing monosaccharide residue was a galactose residue. On methanolysis of the methylated fraction D, 2,3,4,6-tetra-O-methyl-D-Man, 2,4,6-tri-O-methyl-D-Gal, and 4,6-di-O-methyl-D-GalN were identified, i.e., the main oligosaccharide in this fraction was the disaccharide D-Man1→3-D-Gal. Oligosaccharide fractions B and C were more complex oligosaccharide mixtures (Table 2).

Summarizing all the results obtained from qualitative and quantitative monosaccharide analysis, methylation, Smith degradation, and partial hydrolysis, it may be assumed that the O-specific polysaccharide of *Y. enterocolitica* serovar 0:8 is constructed of residues of L-fucose, 6-deoxy-D-gulose, D-mannose, D-galactose, and an aminosugar — probably D-galactosamine (since there have been no reports on the inclusion of the latter in the core) — in equimolar ratio. The other monosaccharides — D-glucose, D- and L-glycero-D-mannoheptoses, and D-glucosamine — are present as components of the core and the accompanying glucan. The O-specific chains have a low degree of polymerization which is comparable with that of the core oligosaccharide (this explains the difficulty of their separation and the absence of specific cleavage in the production of oligosaccharides).

It is obvious from the methylation results that the L-fucose and 6-deoxy-D-gulose residues are located in side chains (the presence of 2,3,4-tri-O-methyl-L-Fuc and of 2,3,4-tri-O-methyl-6d-D-Gul in the hydrolysis products of the methylated LPSs and methylated PS). Their complete disappearance after only the first periodate oxidation step confirmed this. The mannose residues are present in the main polysaccharide chain and are bound in positions 3 and 4, a proof of which is the 2,6-di-O-methyl-D-Man in methanolysates of the methylated LPSs and methylated PS. The increase in the amount of 2,3,6-tri-O-methyl-D-Man in a hydrolysate of the methylated PS, as compared with the LPSs (this can be seen on identification of these compounds in the form of the corresponding polyol acetates) indicates that in the main chain the mannose residues are linked by 1→4 bonds. In addition to this, after the first Smith degradation, when the 6-deoxyhexose residues were completely oxidized, the analysis of the methyl ethers from the methylated polysaccharide showed that the 2,6-di-O-methyl-D-Man had disappeared, the 2,3,6-tri-O-methyl-D-Man had remained, and the amount of 2,3,4,6-tetra-O-methyl-D-Man (formed from the nonreducing end of the O-specific chain) had increased. Thus, one of the 6-dioxyhexose residues, namely the 6-deoxy-D-glucose residue, was attached to the mannose in

TABLE 2. Oligosaaccharide Fractions Obtained from the Lipopolysaccharides of *Y. enterocolitica* Serovar 0.8

Oligosaccharide fractions	$[\alpha]_{578}^{20}$	R_{Gal}	Fuc	6d-Gul	Man	Gal	Glc	Hep	HexN	Oligosaccharides determined from their mass spectra
A	+6	0,86	—	0,2	0,2	0,4	10,	—	Tr.	1. Hex1→4Hex 2. Hex1→HexN 3. 6d-Hex→Hex→Hex
B	+26	0,54	Tr.	0,5	1,0	1,0	0,4	0,3	+ (GalN)	1. Hex1→4Hex 2. Hex1→3Hex 3. Hex1→Hep 4. 6d-Hex1→3Hex1→→3Hex Hex1→3Hex ↑ 6d-Hex 5. Hex1→Hex1→Hex 6. Hex1→Hex1→HexN
C	+34	0,48	0,2	0,2	1,0	1,0	0,3	Tr.	+	1. Hex1→4Hex 2. Hex1→3Hex 3. Hex1→Hep 4. 6d-Hex1→Hex1→→Hex 5. Hex1→Hex1→Hex 6. Hex1→Hex1→HexN
D	+20	0,61	Tr.	0,1	1,0	1,4	0,3	—	+ (GalN)	1. Hex1→3Hex 2. 6d-Hex1→Hex1→→Hex 3. Hex1→Hex1→HexN

Note. R_{Gal} values were determined on Filtrak-50 paper in the butan-1-ol—pyridine—water (6:4:3) system.

position 3. A proof of this is the fact that in a hydrolysate of the methylated polysaccharide fraction with a low level of the 6-deoxy-D-gulose (L-Fuc:6d-D-Glu:D-Man+D-Gal:D-Glc: Hep_{1+2}, as 16:4:24:25:31, respectively), 2,3,6-tri-O-methyl-D-Man was present in considerable amount with an appreciable decrease in the amount of 2,6-di-O-methyl-D-Man.

The presence of 4,6-di-O-methyl-D-Gal in the methanolysates of the methylated LPSs and methylated PS indicated that the D-galactose residue is glycosylated in positions 2 and 3 — in position 3 in the main chain — while the 6-deoxyhexose (l-fucose) substitutent is attached in position 2 to form a side chain. The appearance of 2,4,6-tri-O-methyl-D-Gal after the first Smith degradation when the fucose residue had been oxidized completely, and also the identification of the disaccharide D-Manl→3-D-Gal in the oligosaccharide fraction D demonstrated this.

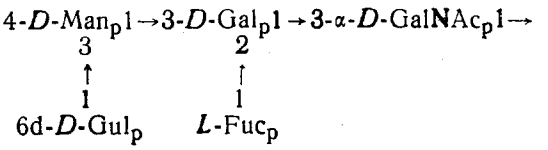
The appearance of 2,4,6-tri-O-methyl-D-Man after the periodate oxidation of the polysaccharide was due to the partial oxidation of the 1→3-bound residues of both core heptoses. After the second oxidation, the mannose residues of the O-specific chains had disappeared completely and only the mannose form from the heptoses remained.

Both the glucosamine and the galactosamine residues are included in the main chain by 1→3 bonds. A proof of this was the identification of 4,6-di-O-methyl-D-GlcN and of 4,6-di-O-methyl-D-GalN both in the initial LPSs and PS and in the products of their oxidation, i.e., their resistance to double Smith degradation, the galactosamine residue probably being present in the O-specific chain and the glucosamine residue in the core. All the monosaccharide residues are present in the pyranose form.

The ^{13}C NMR spectra of the initial polysaccharide (PS) and of the modified polysaccharides OPS-I and OPS-II (after two successive Smith degradations) could not be interpreted completely because of their complexity (very large number of signals). Thus, in the region of resonance of the anomeric carbon atoms in the spectrum of the PS alone, a sequence of 10 signals was observed. Signals with chemical shifts of 15.7 and 16 ppm in the spectrum of the initial PS belonged to the C atoms of the methyl groups of 6-deoxyhexoses (6-d-D-Gul and L-Fuc, respectively), and they disappeared completely even in the spectrum of the OPS-I after the first oxidation. The presence in the spectrum of the PS of the signals of two acetyl groups (22.6 and 23.5, and also 174.4 and 175.4 ppm) and of two carbon atoms linked to nitrogen (49.8 and 55 ppm) showed that it contained N-acetylglucosamine and N-acetylgalactosamine residues, and they were retained after oxidation in the spectra of OPS-I and OPS-II, i.e.,

they were glycosylated in positions 3. Since the question of the configuration of the anomeric centers of the aminosugars is answered by the magnitude of the chemical shift of the C2 atom on comparing literature figures [17] it can be stated that the N-acetyl-glucosamine has the β-configuration (55 ppm), and the N-acetylglucosamine the α-configuration (49.8 ppm).

On the basis of what has been said above, the following structure can be suggested for the repeating unit of O-specific polysaccharide of *Yersinia enterocolitica* serovar 0:8:



EXPERIMENTAL

Descending chromatography of Filtrak FN-12 and FN-15 papers was performed in the butan-1-ol—pyridine—water (6:4:3, by volume) system, the monosaccharides being detected with an alkaline solution of silver nitrate 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) on Toyopearl HW-50F (2.1 × 70 cm) in water, the fractions being analyzed by the phenol/sulfuric acid method.

GLC was performed on a Pyr-Unicam 104 chromatography (United Kingdom) with a flame-ionization detector using glass columns (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 being argon at a rate of 60 ml/min. The polyol acetates derived from the monosaccharides were analyzed on columns A and B in the temperature interval of 175-225°C (5°C/min); the acetates of the methyl glucosides and of the polyols derived from the partially methylated monosaccharides, on column A from 110 to 225°C (5°C/min); and the completely-methylated oligosaccharide fractions on columns C in the temperature interval of 200-300°C (5°C/min). GLC-mass spectrometry was performed on a LKB-9000s instrument using columns with the same phases.

^{13}C NMR spectra of the polysaccharides were taken on a Bruker-Physik WM-250 instument 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 at 20°C.

The microorganism *Y. enterocolitica* serovar 0:8 (strain 161) was obtained from the International Yersinia Center (Paris, Prof. H. H. Mollaret). The cells were grown in a synthetic medium described in [6]. The yield of an acetone powder of the microbial mass was 1.0 g per 1 liter of synthetic medium. The LPSs were isolated by extraction with 45% of aqueous phenol and were purified by three ultracentrifugations at 105,000g [7]. The LPSs were lyophilized, the yield amounting to 1.0-2.0% on the dry weight of the cells.

The complete acid hydrolysis of the LPSs (10 mg) and that of the PS (5 mg) were carried out with 0.5 N trifluoroacetic acid or 2 N HCl at 100°C for 3.5 h. The solution were evaporated several times with methanol. The monosaccharides were identified by the paper chromatography and GLC of the corresponding polyol acetates.

Mild Acid Hydrolysis of the LPSs. The LPSs (1 g) were hydrolyzed with 1% acetic acid (100 ml) in a boiling water bath for 1.5 h. The lipid A that precipitated was separated by centrifugation (15,000 rpm, 20 min). The supernatant was lyophilized and was then separated by gel filtration on Sephadex G-50. A glucan (16 mg) and the polysaccharide (410 mg) were isolated. The latter was rechromatographed on Sephadex G-25, giving the polysaccharide fraction (335 mg), $[\alpha]_{578}^{20}$+47°, containing the O-specific polysaccharide and the core. It was used for the subsequent structural investigations. In addition, a mixture of mono- and oligosaccharides + KDO (70 mg) was obtained. Part of the polysaccharide (100 mg) was chromatographed on a column of Toyopearl HW-50F in an attempt to separate the O-specific polysaccharide from the core. The polysaccharide so obtained was identical with the initial material (85 mg).

Analysis by the Methylation Method. Samples of the LPSs and their fragments (5-10 mg) previously dried at 60°C over P_2O_5 were 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 [9]. The completely methylated compounds (half) were heated in methanolysis mixture (1 N HCl in methanol, 100°C, 3h) and evaporated several times with methanol, and the residue was actylated with acetic anhydride in pyridine (1:1, 0.4 ml, 100°C, 30 min) and investigated by GLC and GLC-MS. The second half of the methylated compounds were subjected to formolysis (0.5 ml of 90% HCOOH, 100°C, 3 h), the reaction mixture was evaporated to dryness, and the residue was hydrolyzed with 0.13 M sulfuric acid (0.5 ml, 100°C, 12 h). The resulting mixture was 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 1.

Smith Degradation. The polysaccharide (150 mg) was treated with a 0.1 M solution of sodium metaperiodate in water (10 ml) at 20°C for 48 h in the dark, and then, with cooling, sodium tetrahydroborate (300 mg) was added to the solution in portions and after 21 h, the mixture was acidified by acetic acid to pH 5 and was chromatographed on a column of Sephadex G-15 in pyridine—acetate buffer. The polyalcohol obtained was kept in 0.5 N HCl (10 ml) at 20°C for 72 h. Then this solution was subjected to gel filtration on Sephadex G-15, the modified polysaccharide OPS-1 was isolated (50 mg, $[\alpha]_{578}^{20}$ +79°), and this was subjected to the same treatment again, giving the doubly oxidized polysaccharide OPS-II (30 mg, $[\alpha]_{578}^{20}$ +64).

Partial Hydrolysis. The LPS (600 mg) was hydrolyzed with 0.25 N sulfuric acid (60 ml) in a boiling water bath for 30 min in a flask with a reflux condenser. The precipitate of lipid A was separated off by centrifugation (15,000 rpm, 20 min). The solution was neutralized with Dowex-1 (HCO_3^- form), concentrated to small volume, and precipitated with a fivefold volume of ethanol. The precipitate was separated by centrifugation and was dissolved in a small volume of water, and the solution was lyophilized (73 mg). The ethanolic solution was evaporated to a syrup, giving a mixture of oligosaccharides (154 mg). The oligosaccharides were isolated with the aid of preparative paper chromatography of the ethanolic extract. As a result, four main oligosaccharide fractions — A, B, C, and D (3, 3.5, 3, and 4 mg, respectively), were obtained and their monosaccharide compositions were established after acid hydrolysis (0.5 N CF_3COOH, 100°C, 3 h) with the aid of GLC (Table 1). In parallel with the oligosaccharide fractions, 6-deoxy-D-gluose (16 mg, $[\alpha]_{578}^{20}$ −31°), L-fucose (4 mg, $[\alpha]_{578}^{20}$ −63°), and D-mannose (5 mg, $[\alpha]_{578}^{20}$ +9°) were isolated.

CONCLUSIONS

The lipopolysaccharides have been isolated from the microbial mass of *Yersinia enterocolitica*, serovar 0:8 (strain 161). The polysaccharide has been obtained by mild acid hydrolysis followed by gel filtration on Sephadexes of various types, and its qualitative and quantitative monosaccharide compositions have been established.

On the basis of the results of methylation, of two successive mass degradations, of partial hydrolysis, of chromato-mass spectrometry, and of ^{13}C NMR, a structure has been suggested for the repeating unit of the O-specific chain of the lipopolysaccharides under investigation.

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STUDY OF THE KINETICS OF THE EXTRACTION OF FLAVONOIDS FROM PLANT RAW MATERIAL

I. EXTRACTION OF RUTIN FROM *Sida hermaphrodita*

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The influence of the concentration of ethanol (50, 60, and 70%, of the degree of grinding of the plant raw material, and of the time of maceration, on the yield of rutin from the epigeal part of *Virginia sida*, family Malvaceae has been studied. It has been established that the highest yield of rutin can be obtained from raw material ground to 2.5-3.0 mm with the use of 70% ethanol and a time of maceration of 12 h. In an investigation of the influence of the concentration of ethanol and the time of steeping on the yield of rutin from raw material ground to 2.5-2.0 mm, regression equations for 50, 60, and 70% ethanolic extractions have been derived.

Questions of the theory and practice of the extraction of raw materials for obtaining medicinal preparations — tinctures and extracts — have been discussed in detail in the literature [1], but the kinetics of the extraction of flavonoids has been little studied [2, 3], although a number of these compounds are used as drugs [4].

We have established by preliminary investigation of some plants of the Malvaceae family that when mucilages are present in the plants the extraction of flavonoids is inhibited. In order to determine the optimum conditions for the extraction of flavonoids we have studied the influence of the degree of grinding of the raw material, the time of steeping, and the concentration of ethanol on the yield of rutin from the epigeal part of *Sida hermaphrodita* Rusby (Virginia sids) in which, according to our results, up to 5% of rutin may be present [5].

Table 1 gives the results on the influence of the degree of grinding of the raw material on the yield of rutin as functions of the time of steeping and of the concentration of ethanol.

TABLE 1. Amounts of Rutin in Extracts as Functions of the Degree of Grinding of the Raw Material, the Time of Steeping, and the Concentration of Ethanol*

Concentration of ethanol, %	Degree of grinding of the raw material, mm	Time of steeping, h				
		1	3	6	12	24
50	1	1,64	1,2	1,62	1,54	1,61
60	1	1,55	1,55	1,50	2,40	1,96
70	1	1,47	1,50	1,55	1,96	2,00
50	2	1,48	1,44	2,16	2,05	1,64
60	2	2,30	2,16	2,22	1,96	1,9
70	2	2,05	2,10	2,22	2,46	2,56
50	3	1,47	1,44	2,16	1,56	2,10
60	3	2,16	2,10	2,16	2,10	2,12
70	3	3,00	2,16	2,10	2,35	2,27
50	7	0,61	1,03	1,34	1,43	1,35
60	7	1,24	1,32	1,44	1,89	1,80
70	7	1,60	1,19	1,42	1,48	1,37

*Sieves Nos. 10, 20, 30, 50, and 70 (GOST [State Standard] 214-57) were used [7].

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