MONOSACCHARIDE COMPOSITION OF THE O-SPECIFIC POLYSACCHARIDE OF THE SIDE CHAINS OF THE LIPOPOLYSACCHARIDE OF *Yersinia pseudotuberculosis* SEROVAR VB

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It has been shown by ^{13}C NMR spectroscopy that the O-specific polysaccharide from *Yersinia pseudotuberculosis* serovar VB (strain R2) consists of regularly repeating pentasaccharide units. D-Galactose, D-mannose, and L-fucose residues have been identified in the polysaccharide. The presence of 6-deoxy-L-altrose has been shown by paper, gas-liquid, and thin-layer chromatographies, mass spectrometry, and ^{1}H and ^{13}C NMR spectroscopy, and the L configuration of the monosaccharide has been determined polarimetrically. It has been shown that the repeating unit of the polysaccharide contains two L-fucose residues and one residue each of 6-deoxy-L-altrose, D-mannose, and D-galactosamine.

Previously [1], in the lipopolysaccharide of *Y. pseudotuberculosis* serovar VB, strain R2, we detected a monosaccharide which was not identified. In the present paper we give information on the characterization of the polysaccharide of the O-specific side chains of the lipopolysaccharide, the determination of its molecule structure, and the identification of the monosaccharide mentioned as 6-deoxy-L-altrose. Ellwood and Kirk [2] have identified 6-deoxy-L-altrose as a component of a bacterial polysaccharide in a study of an untyped strain of *Yersinia enterocolitica* isolated from a human subject. 6-Deoxy-L-altrose has not been found in any of the strains of *Y. pseudotuberculosis* studied previously [3].

The polysaccharide of the O-specific side chains was isolated as the result of hydrolysis of the lipopolysaccharide with 1% acetic acid and gel filtration on Sephadex G-50.

The specific polysaccharide $[\alpha]_{578}^{20}$ + 4° (c 0.3; water) issued immediately after the free volume of the column, was homogeneous in molecular weight, and gave a symmetrical peak on analytical ultracentrifugation.

In the ^{13}C NMR spectrum of the O-specific polysaccharide five signals were observed in the region of absorption of anomeric C-atoms at 105.5, 102.4, 102.1, 98.1, and 98.4 ppm with equal integral intensities. Consequently, the polysaccharide is constructed of regularly repeating pentasaccharide units. The presence of three signals in the spectrum at 18.3, 17.15, and 16.0 ppm relating to the C-atoms of methyl groups of 6-deoxysugars [4] and two signals with chemical shifts of 61.9 and 61.74 ppm relating to the C-atoms of hydroxymethyl groups [4] showed that the repeating unit consisted of three residues of 6-deoxysugars and two hexose residues.

In a homogeneous of the polysaccharide, paper chromatography in system 1 showed the presence of a monosaccharide with R_{Rha} 1.14 and of fucose, mannose, and galactosamine, and the presence of the latter was confirmed by amino acid analysis (10.3%). The hydrolysate obtained was subjected to deamination [5] with subsequent reduction by sodium tetrahydroborate and acetylation. Fucose, 2,5-anhydrotalose, and mannose were identified in a ratio of 2.9:1.0:1.0. In its retention time, the unidentified monosaccharide coincided with fucose, which permitted the assumption that it was a 6-deoxyhexose.

Paper chromatography and paper electrophoresis of the hydrolysates of the polysaccharide and the lipopolysaccharide permitted the isolation of the monosaccharides, and their optical rotations were determined, showing the L configuration of the fucose and the D configurations of the mannose and of the galactosamine.

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TABLE 1. Chemical Shifts of the ^{13}C Carbon Atoms of Methyl Glycosides of 6-Deoxy-L-altrose

Compound	C-1	C-2	C-3	C-4	C-5	C-6	OMe
Methyl 6-deoxy-α-L-altrofuranoside	108,9	88,0*	76,7	81,5*	67,4	18,2	55,4
Methyl 6-deoxy-β-L-altrofuranoside	102,6	85,5*	77,2	75,8*	68,9	18,2	55,9
Methyl 6-deoxy-α-L-altropyranoside	102,2	70,7	70,7	70,7	66,7	17,0	56,1
Methyl 6-deoxy-β-L-altropyranoside	100,3	71,1	70,4	70,4	70,4	17,9	57,3

*Assignment of the signals ambiguous.

The preparatively isolated unidentified monosaccharide was studied by chromato-mass spectrometry in the form of the corresponding acetylated polyol, and the m/z values of the main fragments of the spectrum — 43, 99, 103, 115, 128, 170, 187, 231, 289, and 303 — confirmed that the monosaccharide was a 6-deoxyhexose.

On thin-layer chromatography in system 3, the monosaccharide had R_{Rha} 1.28, which agrees with literature figures [6] for 6-deoxy-L-altrose. In its chromatographic behavior on paper in system 2 and its electrophoretic mobility on paper in borate buffer, likewise, the monosaccharide was similar to 6-deoxyaltrose [6].

The optical rotation of the 6-deoxyhexose, $[\alpha]^{20}$ — 20°; (c 0.5, water) was close to the angle of rotation found for 6-deoxy-L-altrose [2].

In the PMR spectrum (ν = 360 MHz) of the methyl glycoside of the presumed 6-deoxy-L-altrose that had been isolated preparatively four doublet signals of anomeric protons were observed with δ_1 = 4.93 ppm, $J_{1,2}$ = 1.5 Hz; δ_2 = 4.78 ppm, $J_{1,2}$ = 3.6 Hz; δ_3 = 4.77 ppm, $J_{1,2}$ = 1.8 Hz; δ_4 = 4.61 ppm, $J_{1,2}$ = 2.7 Hz, and doublet signals with an SSCC of J = 6 Hz in the relatively strong magnetic field with δ_1 = 1.24 ppm, δ_2 = 1.25 ppm, δ_3 = 1.31 ppm, and δ_4 = 1.32 ppm. This showed the presence in solution of α,β-pyranose and α,β-furanose forms, and also the presence of a 6-deoxy unit [7]. The small SSCCs of the signals of the anomeric protons, $J_{1,2} < 4$ Hz, showed the axial orientation of the group at C-2 [8]. At the same time, since the doublet at δ_1 = 4.93 ppm, $J_{1,2}$ = 1.5 Hz is a low-field signal, it relates to the equatorial anomeric proton of the α form [7] and for it a value $J_{1,3}$ = 0.7 Hz was observed, which is characteristic for a zigzag-shaped (W-shaped) configuration of the interacting protons [7]. This means that the OH group at C-3 for this form of the monosaccharide is equatorial. This is also confirmed by the value of the SSCC for the H-3 proton for the forms characterized by the anomeric protons, δ = 4.97 ppm and δ = 4.61 ppm, $J_{3,2}$ = 2.44 Hz, $J_{3,4}$ = 5.13 Hz and $J_{1,2}$ = 4.8 Hz, $J_{3,4}$ = 3.1 Hz. Irradiation of the methyl protons permits the isolation of the signals of proton at C-5 and showed that the signals of the proton at C-5 had SSCCs of $J_{4,5}$ = 8.1 Hz for the pyranose forms and $J_{4,5}$ = 5.1 Hz for the furanose forms, which is characteristic for the axial—axial interaction of vicinal protons. Thus, the PMR spectrum confirmed that the monosaccharide under investigation was 6-deoxyaltrose.

This conclusion is also confirmed by the ^{13}C NMR spectrum of the methyl glycoside of the monosaccharide isolated preparatively. The small difference in the chemical shifts of the anomeric C-atoms of the pyranose form shows the axial orientation of the OH group at C-2. A comparison of the magnitudes of the chemical shifts of the C-atoms in the spectrum of altrose, taking into account the replacement of a hydroxymethyl group by a methyl group [9], and of the methylglycoside of the monosaccharide under investigation showed good agreement. We carried out an assignment of the signals by using figures from the spectrum of altrose and differences in the ratios of the anomeric forms. (Table 1).

The acetylated methyl glycoside of the 6-deoxy-L-altrose isolated preparatively was studied by chromato-mass spectrometry, which showed that the first peak that issued contained 6-deoxy-L-altrofuranoside, the second a mixture of the pyranose and furanose forms, and the third the 6-deoxy-L-altropyranoside.

After methanolysis of the polysaccharide followed by acetylation, chromato-mass spectrometry showed the presence of four peaks relating to 6-deoxyhexoses. The retention time of

the first of them coincided with that of the 6-deoxy-L-altrofuranoside, which enabled the ratio between the 6-deoxy-L-altrofuranose and L-fucose residues in the polysaccharide to be determined as 1 : 2. On the basis of the facts given above, it may be concluded that the polysaccharide of the O-specific side chains is composed of a pentasaccharide repeating unit containing one residue each of 6-deoxy-L-altrose, D-mannose, and D-galactosamine and two of L-fucose.

EXPERIMENTAL

Descending paper chromatography of the monosaccharides was performed in the following solvent systems: 1) butan-1-ol—pyridine—water (6 : 4 : 3, by volume); and 2) toluene—butan-1-ol (1 : 2)—water on Filtrak FN-3 and Whatman No. 1 papers. The monosaccharides were detected with an alkaline solution of silver nitrate and with aniline hydrogen phthalate.

Paper electrophoresis was performed in 0.025 M pyridine-acetate buffer (pH 4.5) at 28 V/cm for 90 min and in borate buffer (pH 10.4) for 4 h 15 min.

Thin-layer chromatography was performed on LSL_{254} 5/40 silica gel (Czechoslovakia) in system 3 (ethyl acetate—isopropanol—methanol (70 : 15 : 15)); the monosaccharides were detected with concentrated sulfuric acid.

Gas-liquid chromatography was performed on a Pye Unicam 104 chromatograph with a flame-ionization detector using glass columns packed with the phase QF-1 (3%) on Gas-Chrom Q (100-120 mesh) at a rate of flow of argon of 60 ml/min. The monosaccharides were analyzed in the form of the acetates of the corresponding polyols with programming of the temperature from 175 to 225°C, and the acetates of the methyl glycosides with programming from 110 to 225°C at the rate of 5 deg/min.

Chromato-mass spectrometry was performed on a LKB-9000 S instrument using a column containing the phase QF-1.

Optical rotations were determined on a Perkin-Elmer 141 polarimeter. Amino acid analyses were performed on a LKB-Biocal 3201 analyzer in 45 × 0.9 cm columns packed with JEOL LC-R2 resin. The homogeneity of the polysaccharides was determined on a 6-120 MOM analytical ultracentrifuge.

^1H and ^{13}C NMR spectra were recorded on a HX-360 instrument (ν = 90.55 MHz) in D_2O using methanol as internal standard; chemical shifts are given in parts per million after recalculation by means of the relation $\delta_{TMS} = \delta_{MeOH} + 49.6$ ppm (for ^{13}C NMR).

Isolation of the Specific Polysaccharide. The *Y. pseudotuberculosis* VB-R2 lipopolysaccharide (strain obtained from Tsubakura, Japan) (900 mg) was hydrolyzed with 1% acetic acid (90 ml) in the boiling water bath for 3 h. Lipid A (yield 241 mg) was isolated by ultracentrifugation at 105,000 × g for 1 h. The supernatant liquid was lyophilized (yield 358 mg). Gel chromatography of the hapten fraction obtained was carried out on Sephadex G-50 (column 65 × 1.6 cm) in pyridine-acetate buffer at pH 5.6 [pyridine—acetic acid—water (10 : 4 : 986, by volume)]. Fractionation was monitored by the phenol/sulfuric acid, the yield of the first fraction being 80 mg and of the second 143 mg.

Acid Hydrolysis. The hydrolysis of 50 mg of the polysaccharide was carried out with 5 ml of 4 N HCl at 100°C for 4 h, and the acid was eliminated by repeated evaporation with methanol. The hydrolysate was used for the preparative isolation of the monosaccharides, part of it (2 mg) being subjected to deamination [5].

Methanolysis. A mixture of 3 mg of the polysaccharide and 2 ml of 2% HCl in methanol, was heated in a sealed tube for 6 h, the HCl was eliminated by evaporation with methanol, and the 6-deoxy-L-altrose isolated preparatively was subjected to methanolysis with the same methanolysis mixture for 2 h.

SUMMARY

The monosaccharide composition of the polysaccharide of the O-specific side chains of the lipopolysaccharide of *Yersinia pseudotuberculosis* serovar VB have been studied. The presence of 6-deoxy-L-altrose residues has been shown by paper, gas-liquid, and thin-layer chromatographies, mass spectrometry, and ^1H and ^{13}C NMR spectroscopy, and its L configuration has been determined polarimetrically.

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POLYSACCHARIDES OF *Ungernia*.

X. PECTINS FROM THE LEAVES OF *Ungernia sewerzowii*

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The characteristics of the pectin from the leaves of *Ungernia sewerzowii* are given. The pectin isolated consists of residues of: galacturonic acid — 51%; rhamnose — 23.6%; arabinose — 4.9%, xylose — 1.3%; glucose — 4.1%; and galactose — 15.3%. $[\alpha]_D^{25}$ + 206° (c 0.5; water, OCH_3 — 5.4%. Partial hydrolysis of the pectin gave a galacturonan containing only galacturonic acid residues. On the basis of the results of periodate–silver nitrate oxidation and methylation it was shown that the galacturonic acid residues in the galactone are linked by α-1 → 4-glycosidic bonds.

It has been found previously [1] that the predominating polysaccharides of the leaves of *Ungernia sewerzowii* (Rgl.) B. Fedsch. are pectin substances, and those of the bulbs are reserve polysaccharides: a natively acetylated mannan and starch. We have determined the amount of carbohydrates in the leaves of *Ungernia sewerzowii* gathered on April 16, 1976 in Galvasae, Tashkent province. The polysaccharides were extracted successively from one sample of air-dry material: first the water-soluble polysaccharides [2], and then the pectin substances [3] and the hemicelluloses (Table 1).

The pectin substances have been studied in more detail. The pectins isolated consisted of a cream-colored powder readily soluble in water, $[\alpha]_D^{25}$ + 206° (c 0.5; water). The amount of galacturonic anhydride determined by a standard method [4] was 51%, and the amount of OCH_3 5.4%. The percentages of the neutral monosaccharides were as follows: rhamnose, 23.6; arabinose, 4.9; xylose, 1.3; glucose, 4.1; and galactose, 15.3. The galacturonic acid, which was isolated in the pure state, was identified by oxidation to mucic acid with mp 215-216°C [5].

The quantitative characteristics of the pectin obtained by a titrimetric method [6] were (%): free carboxy groups, K_f — 11.5; methoxylated carboxy groups K_e — 7.1; degree of methoxylation, λ — 38.

The IR spectrum of the pectin contained the absorption bands that are characteristic for other pectins [7]: 3420, 2950, 1750, 1640, 1120, 1020, and 840 cm^{-1}.

The molecular weight, determined viscosimetrically [8] was 54,000 a.u., and that calculated from the sedimentation constant was 49,000 a.u.

The periodate oxidation of the pectins was carried out in a neutral medium. The consumption of sodium periodate amounted to 0.53 mole per mole of anhydrohexose per unit. On Smith

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