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 $^1H$ 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 $[\alpha]_D^{20} + 4^\circ$ (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 $R_{RHS}$ 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.
TABLE 1. Chemical Shifts of the $^{13}$C Carbon Atoms of Methyl Glycosides of 6-Deoxy-\(\beta\)-altrose

<table>
<thead>
<tr>
<th>Compound</th>
<th>C-1</th>
<th>C-2</th>
<th>C-3</th>
<th>C-4</th>
<th>C-5</th>
<th>C-6</th>
<th>OMe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl 6-deoxy-(\alpha)-altrofuranoside</td>
<td>108.9</td>
<td>98.0*</td>
<td>78.7</td>
<td>81.5*</td>
<td>67.4</td>
<td>18.2</td>
<td>55.4</td>
</tr>
<tr>
<td>Methyl 6-deoxy-(\beta)-altrofuranoside</td>
<td>102.6</td>
<td>85.5*</td>
<td>77.2</td>
<td>75.8*</td>
<td>69.9</td>
<td>18.2</td>
<td>55.9</td>
</tr>
<tr>
<td>Methyl 6-deoxy-(\alpha)-altropyranoside</td>
<td>102.3</td>
<td>70.7</td>
<td>70.7</td>
<td>70.7</td>
<td>66.7</td>
<td>17.0</td>
<td>56.1</td>
</tr>
<tr>
<td>Methyl 6-deoxy-(\beta)-altropyranoside</td>
<td>100.3</td>
<td>71.1</td>
<td>70.4</td>
<td>70.4</td>
<td>70.4</td>
<td>17.9</td>
<td>57.3</td>
</tr>
</tbody>
</table>

*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-\(\beta\)-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, \([\alpha]_{D}^{20} = -20^\circ\); (c 0.5, water) was close to the angle of rotation found for 6-deoxy-\(\beta\)-altrose [2].

In the PMR spectrum (\(\nu = 360\) MHz) of the methyl glycoside of the presumed 6-deoxy-\(\beta\)-altrose that had been isolated preparatively four doublet signals of anomeric protons were observed with \(\delta = 4.93\) ppm, \(J_{1,2} = 1.5\) Hz; \(\delta = 4.78\) ppm, \(J_{1,2} = 3.6\) Hz; \(\delta = 4.77\) ppm, \(J_{1,2} = 1.8\) Hz; \(\delta = 4.61\) ppm, \(J_{1,2} = 2.7\) Hz, and doublet signals with an SSCC of \(J = 6\) Hz in the relatively strong magnetic field with \(\delta = 1.24\) ppm, \(\delta = 1.25\) ppm, \(\delta = 1.31\) ppm, and \(\delta = 1.32\) ppm. This showed the presence in solution of \(\alpha,\beta\)-pyranose and \(\alpha,\beta\)-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 \(\delta = 4.93\) ppm, \(J_{1,2} = 1.5\) Hz is a low-field signal, it relates to the equatorial anomeric proton of the \(\alpha\) form [7] and for it a value \(J_{1,2} = 0.7\) 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, \(\delta = 4.97\) ppm and \(\delta = 4.61\) ppm, \(J_{3,4} = 2.44\) Hz, \(J_{3,4} = 5.13\) Hz and \(J_{3,4} = 4.8\) 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 \(J_{5,6} = 8.1\) Hz for the pyranose forms and \(J_{5,6} = 5.1\) 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-\(\beta\)-altrose isolated preparatively was studied by chromato-mass spectrometry, which showed that the first peak that issued contained 6-deoxy-\(\alpha\)-altrofuranoside, the second a mixture of the pyranose and furanose forms, and the third the 6-deoxy-\(\beta\)-altropyranoside.

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