Structure of O-Specific Side Chains of Lipopolysaccharides from Yersinia pseudotuberculosis

KURT SAMUELSSON, BENGT LINDBERG, AND ROBERT R. BRUBAKER

Institutionen för Organisk Kemi, Stockholms Universitet, S-113 27 Stockholm, Sweden, and Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan 48823

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Lipopolysaccharide prepared from cells of Yersinia (Pasteurella) pseudotuberculosis of serogroups I, II, III, IV, and V is known to contain the 3,6-dideoxyhexose (DDH) paratose, abequose, paratose, tyvelose, and asaclyrose in its respective O-specific side chains. Lipopolysaccharides or lipid-free polysaccharides of all of the 10 known serogroups and subgroups were subjected to methylation analysis and determined as alditol acetates by gas-liquid chromatography and mass spectrometry. The results indicated that the O-specific side chains of nine serotypes are composed of oligosaccharide repeating units in the form of four alternative general structures in which a terminal DDH may vary.

These structures are DDH $\alpha_1^3$ 6-deoxy-D-manno-heptose $\alpha_4^4$ D-galactose (serogroups IA, IIA, and IVB), DDH $\alpha_1^3$ D-mannose $\alpha_3^3$ L-fucose (serogroups IB and IIB), and two configurations similar to the latter except that the 4-position of L-fucose was either linked to the D-mannose residue (serogroups VA and VB) or to the DDH residue (serogroups III and IVA). In contrast, O-groups in lipopolysaccharide of the newly discovered serogroup VI contained the DDH colitose and 2-acetamido-2-deoxy-D-galactose. Accordingly, all five known types of DDH have now been detected in lipopolysaccharides of Y. pseudotuberculosis. The sugar 6-deoxy-D-manno-heptose, present in O-specific side chains of serogroups IA, IIA, and IVB, has not yet been reported to occur elsewhere in nature.

Isolates of Yersinia (Pasteurella) pseudotuberculosis were classified by Thal (16) into five major serogroups, four of which were subsequently divided into subgroups (17). The O-specific side chains comprising the lipopolysaccharides of the major serogroups I, II, III, IV, and V were shown by Davies (3, 4) to contain 3,6-dideoxy-D-ribo-hexose (paratose), 3,6-dideoxy-D-xylu-hexose (abequose), paratose, 3,6-dideoxy-D-arabino-hexose (tyvelose) and 3,6-dideoxy-L-arabinose (ascarlyose), respectively. Distinctions in the structure of O-specific side chains or different linkages of paratose were assumed to account for the presence of the latter in lipopolysaccharides of cells of serogroups I and III. The composition of the O-specific side chains in lipopolysaccharide from cells of the newly discovered serogroup VI (15) has not yet been reported, but an antigenic relationship exists with serogroup 055 lipopolysaccharide of Escherichia coli (14) which is known to contain 3,6-dideoxy-D-xylo-hexose (colitose) (13).

One purpose of the investigation described in this report was to define the putative differences in O-specific side chains which permit the occurrence of paratose in two major serogroups and which account for the existence of subgroups possessing a common 3,6-dideoxyhexose. A second objective was to obtain direct evidence for the presence of colitose in lipopolysaccharide isolated from yersiniae of serogroup VI. We have previously presented tentative structures of O-specific side chains in lipopolysaccharide of serogroups IIA and IIB (7, 8); preliminary studies of some other serogroups have also been noted (9). The results of structural analyses of O-specific side chains from all known serogroups are reported in this paper.

MATERIALS AND METHODS

Bacteria. The strains of Y. pseudotuberculosis used in this study were 1 (serogroup IA), 1B (serogroup IB), 7 (serogroup IIA), 1779 (serogroup IIB), 43 (serogroup III), 32 (serogroup IV), 25 (serogroup VA), R2 (serogroup VB), etc.
and 3 (serogroup VI). These isolates were received through the courtesy of W. Knapp.

**Cultivation.** Bacteria were grown in a medium consisting of 3% NZ amine, type A (Sheffield), 1% D-xylene, and the salt component contained in the medium of Higuchi and Carlin (10); the initial pH was adjusted to 7.2 with 10 N NaOH. After aeration at 26 C in fermentor vessels (model F8 305, New Brunswick) containing 3 liters of medium or in 2-liter flasks containing 400 ml of medium, the cells were collected during the early stationary phase by centrifugation at 27,000 x g for 15 min, washed in 0.033 M potassium phosphate buffer (pH 7.0), suspended in distilled water, and lyophilized.

**Isolation of lipopolysaccharide.** Lyophilized bacteria were thoroughly suspended in distilled water, and lipopolysaccharide was extracted with hot phenol; after phase separation upon chilling and centrifugation, the aqueous layer was exhaustively dialyzed against cold distilled water and contaminating substances were removed by differential centrifugation (13). Final yields of lipopolysaccharide were 0.5 to 1% of the initial dry weight of the bacteria.

**Preparation of polysaccharide.** In some cases (serogroups IIa, IIB, and III), an approximate 0.5% suspension of lipopolysaccharide in 0.5% aqueous acetic acid was maintained at 100 C for 1.5 hr before cooling and concentration (fivefold). The suspension then received 4 volumes of ethanol and was extracted with 5 volumes of hexane; lipid-free polysaccharide was recovered from the ethanol-water phase. In cases where nonsugar components remained in the polysaccharide solution, the latter was further purified by chromatography on Sephadex G-25. Polysaccharide was eluted in a concentrated fraction detectable by optical rotation.

**Sugar content.** Portions of each preparation were subjected to essentially complete hydrolysis with 0.25 M H2SO4 for 12 hr at 100 C. After neutralization and concentration, the component sugars were converted into their alditol acetates and subjected to quantitative and qualitative analysis by gas-liquid chromatography and mass spectrometry (GLC-MS) (1). The alditol acetate derived from 2-acetamido-2-deoxy-D-galactose showed the same mass spectrum and chromatographic mobility (ECNSS-M, 200°, 210°; UCW-98, 200°) as the authentic sample and was chromatographically distinguishable from the corresponding 2-acetamido-2-deoxy-D-glucose derivative.

**Methylation analyses.** Additional portions of each preparation were methylated by the Hakomori procedure (5), hydrolyzed as described above, reduced with borodeuteride, and acetylated; the resulting mixture of alditol acetates was analyzed by GLC-MS (1). The results obtained in these determinations were compared with those observed after mild hydrolysis in 0.25 M H2SO4 for 15 min at 80 C; this treatment selectively cleaved 3,6-dideoxyhexopyranosidic linkages as judged by polarimetric analysis. After hydrolysis under this set of conditions, the component carbohydrates were recovered and subjected to methylation analyses as already described.

**Polarimetric analyses.** Approximate 0.4% solutions of polysaccharides were prepared in 0.25 M H2SO4, and 1 ml was transferred into a 10-cm polarimeter tube maintained at 80 C; optical rotation was determined at intervals. Hydrolysis under this condition was extended for 5 hr, at which time D-mannosyl-L-fucose was prepared for paper chromatography.

**Paper electrophoresis and chromatography.** Neutralized and concentrated partial hydrolysates containing D-mannosyl-l-fucose were subjected to paper electrophoresis in germanate buffer (7) or chromatographed on Whatman 3MM paper with ethyl acetate-acetic acid-water (3:1:1). Partial hydrolysates containing colitose and 2-acetamido-2-deoxy-D-galactose received 4 volumes of ethanol and, after removal of the precipitate by centrifugation, the supernatant fluid was concentrated about 12-fold and chromatographed in the same solvent system on Whatman no. 1 paper.

**Identification of colitose.** The alditol acetate of the 3,6-dideoxyhexose from lipopolysaccharide of serogroup VI showed the same mass spectrum and chromatographic mobility as those of the D-xylodervative (abequose). In contrast, the alditol acetates of the ribo- and arabinono-configurations were easily distinguished by gas chromatography from the xylo-derivative (ECNSS-M column). The negative rotation, [α]D = -50°, of the serogroup VI alditol indicated that the sugar is the L-xylol-configuration (colitose) (12).

**RESULTS**

After complete hydrolysis of the lipopolysaccharides or polysaccharides of the 10 serogroups, the component sugars were converted to alditol acetates and determined by GLC-MS. The results (Table 1) indicate that all preparations except that of serotype VB contained a 3,6-dideoxyhexose. It was evident that the preparations could be separated into three major classes depending upon the presence of 6-deoxy-D-manno-heptose but not L-fucose and D-mannose (serogroups IA, IIA, and IVB), the presence of L-fucose and D-mannose but not 6-deoxy-D-manno-heptose (serogroups IB, IIB, III, IV, VA, and VB), or the absence of all three of these sugars (serogroup VI). It was therefore assumed that these sugars were components of the respective O-specific side chains. Preparations containing 6-deoxy-D-manno-heptose also contained approximately equimolar concentrations of D-galactose, suggesting that in these cases the latter sugar was also a constituent of the O-specific side chains. D-Galactose, in preparations lacking 6-deoxy-D-manno-heptose, and the remaining sugars were therefore assumed to represent components of the core region of the lipopolysaccharides.

Preparations containing 6-deoxy-D-manno-heptose (serogroups IA, IIA, and IVB) were subjected to methylation analysis. It was evi...
dent that all sugars were pyranosidic and that the 3,6-dideoxyhexose residues were terminal (Table 2). The latter are linked to the 3-position of 6-deoxy-D-manno-heptose as judged by the replacement of 6-deoxy-2,4,7-tri-O-methylheptose by 6-deoxy-2,3,4,7-tetra-O-methylheptose upon methylation after selective hydrolysis of 3,6-dideoxyhexopyranosidic linkages. The 2,3,6-tri-O-methyl-D-galactose represents terminal D-galactose which is linked at the 4-position to 6-deoxy-D-manno-heptose and linked (1 \to 3) to D-galactose residues of adjacent O-specific repeating units. Results of polarimetric determinations of polysaccharide of serogroup IIA indicated that all linkages were of the \( \alpha \)-configuration (8). Accordingly, the O-specific side chains have the general structure shown in Fig. 1, where the 3,6-dideoxyhexoses

<table>
<thead>
<tr>
<th>TABLE 1. Sugar content of lipopolysaccharides of the 10 serotypes of Yersinia pseudotuberculosis*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sero-type</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
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</tr>
<tr>
<td>IB</td>
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<td>IIA</td>
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<td>IV</td>
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<tr>
<td>V</td>
</tr>
<tr>
<td>VB</td>
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<tr>
<td>VI</td>
</tr>
</tbody>
</table>

*Individual values refer to percentage of total lipopolysaccharide.

**Weight percentage of total lipopolysaccharide.

<table>
<thead>
<tr>
<th>TABLE 2. Methyl ethers obtained from the hydrolysates of fully methylated (A) and partially hydrolyzed, fully methylated preparations (B) of lipopolysaccharides or polysaccharide from Y. pseudotuberculosis serotypes IA, IIa, and IVB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sugar</strong></td>
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<tr>
<td>----------</td>
</tr>
<tr>
<td>2,4-Tyv</td>
</tr>
<tr>
<td>2,4-Abe</td>
</tr>
<tr>
<td>2,4-Par</td>
</tr>
<tr>
<td>2,3,4,6-G</td>
</tr>
<tr>
<td>2,3,4,6-Gal</td>
</tr>
<tr>
<td>2,3,4,7-6d-Hep</td>
</tr>
<tr>
<td>2,3,4,6,7-Hep</td>
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<tr>
<td>3,4,6-G</td>
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<tr>
<td>2,3,6-Gal</td>
</tr>
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<td>2,4,7-6d-Hep</td>
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<td>2,6-Gal</td>
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<td>2,3,6,7-Hep</td>
</tr>
<tr>
<td>2,3,4,6,7-Hep</td>
</tr>
<tr>
<td>2,4,6-Hep</td>
</tr>
</tbody>
</table>

*Since considerable amounts of the volatile 2,4-di-O-methyl-3,6-dideoxyhexoses and derivatives were lost during the analysis, the molar percentages are given relative to that of total 6-deoxyheptose derivatives, which are assumed to represent all of the 6-deoxyheptose in the sugar analysis.

**2,4-Tyv, 2,4-di-O-methyltyvelose; 2,3,4,6,7d-Hep, 6-deoxy-2,3,4,7-tetra-O-methylheptose, etc.

Retention time of the corresponding alditol acetate on the ECNSS-M column relative to that of 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-d-glucitol.

*Trace.
are paratose, abequose, and tyvelose in sero-

By determining the proportions of 2,6-di-\textit{O}-

Methylation analysis of four preparations

serogroups IA, IIA, and IVB.

linked (1 \to 3) to L-fucose within each O-specific

repeating unit. This finding was in accord with

the detection of \textit{D}-mannosyl-L-fucose upon

paper chromatography of hydrolysates of poly-

saccharide of serogroup IIB (7). The methyla-

tion patterns also demonstrate that L-fucose is

linked (1 \to 2) to \textit{D}-mannonse residues in adja-

cent repeating units. The linkages were of the

\textit{a}-configuration as indicated by results obtained

by polarimetric analysis of polysaccharide of

serogroup IIB (7).

In only two cases was the 3,6-dideoxyhexose

residue linked (1 \to 3) to \textit{D}-mannonse. Accord-

ingly, the structure shown in Fig. 2 is proposed

for these O-groups where the 3,6-dideoxyhexose

is paratose (serogroup IB) or abequose (sero-

group IIB). The average number of repeating

units, as judged by the ratio of 4,6-di-\textit{O}-meth-

yl-\textit{D}-mannonse to 2,4,6-tri-\textit{O}-methyl-\textit{D}-mannonse,

was 0.5 and 1 for the preparation of

serogroups IB and IIB, respectively.

The 3,6-dideoxyhexose residue was linked (1

\to 4) to L-fucose in the O-groups of the remain-

ning two preparations (Fig. 3). In this case the

3,6-dideoxyhexose is paratose (serogroup III)

tyvelose (serogroup IVA). A comparison of

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Sugar & $T_1$ & $T_2$ & Mol\% for serotype & & \\
\hline
 & IB & IIB & III & IVA & \\
\hline
2,4-Tyv & 0.29 & & 5.7 & 8.2 & 6.8 & +
\hline
2,4-Abe & 0.32 & & & & & \\
\hline
2,4-Par & 0.34 & & 5.0 & 12.5 & 11.9 & 8.6
\hline
2,3,4,6-Man & 1.00 & 0.99 & 15.0 & 15.0 & 20.0 & 20.0
\hline
2,4-Fuc & 1.12 & 1.02 & 12.5 & 6.8 & 6.3 & 0
\hline
2-Fuc & 1.67 & 1.43 & & & & \\
\hline
2,3,4,6,7-Hep & 1.68 & 1.68 & 12.6 & 5.3 & 18.4 & 21.9
\hline
3,4,6-Man & 1.95 & 1.82 & & & & \\
\hline
3,4,6-G & 1.96 & 1.83 & 16.2 & 7.2 & 7.2 & 6.6
\hline
2,4,6-Man & 2.09 & 1.90 & 8.4 & 0 & 0.9
\hline
2,3,4,6,7-Hep & 2.17 & 1.90 & 13.1 & 13.1 & & \\
\hline
2,3,6-G & 2.50 & 2.32 & & & 5.2 & \\
\hline
4,6-Man & 3.29 & 2.92 & 8.8 & 0 & 0.7
\hline
2,4-G & 5.1 & 4.21 & & & 2.2 & 0.5
\hline
2,3,6,7-Hep & 5.6 & & 2.4 & 5.9 & 2.5 & 1.9
\hline
2,3,4,6-Hep & 5.9 & 2.4 & 3.2 & & 1.5 & 1.5
\hline
2,4,6-Hep & 12 & 1.7 & 4.8 & 4.4 & 4.6 & 0.5
\hline
\end{tabular}
\caption{Methyl ethers obtained from the hydrolysates of fully methylated (A) and the partially hydrolyzed, fully methylated preparations (B) of lipopolysaccharide or polysaccharide from \textit{Y. pseudotuberculosis} serotypes IB, IIB, III, and IVA}
\end{table}

\* Since considerable amounts of the volatile 2,4-di-\textit{O}-methyl-3,6-dideoxyhexoses and derivatives were lost during the analysis, the molar percentages are given relative to that of total L-fucose derivatives, which are assumed to represent all of the L-fucose in the sugar analysis.

\* See Table 2.

\* Retention time of the corresponding alditol acetate on the OV-225 column relative to that of 1,5-di-\textit{O}-acetyl-2,3,4,6-tetra-\textit{O}-methyl-\textit{D}-glucitol.

\* Trace.
the ratios of tetramethyl ethers of d-glucose and d-mannose indicates that both of these preparations contained about two O-specific repeating units per molecule of lipopolysaccharide. The results of polarimetric analysis verified that all sugars in the O-groups of serogroup III were linked in the a-configuration (Fig. 4).

The results of methylation analyses of the two remaining members of the class containing d-mannose and l-fucose (serogroups VA and VB) are shown in Table 4. In both preparations, 2,3-di-O-methyl-l-fucose but not 2,4-di-O-methyl-l-fucose is observed. Furthermore, the L-fucose content determined during sugar (Table 1) and methylation analyses (Table 4) was significantly higher than that of D-mannose. The ascaryllose present in the O-specific side chains of serogroup VA lipopolysaccharide is probably linked to the d-mannose residue because 2,4,6-tri-O-mannose disappeared and 2,3,4,6-tetra-O-methyl-D-mannose was formed upon mild acid hydrolysis. Curiously, no 3,6-dideoxyhexose was observed in preparations of serogroup VB. These findings suggest that the structure shown in Fig. 5 represents the O-groups in lipopolysaccharide of serogroups VA and VB where the 3,6-dideoxyhexose, when present, is ascaryllose. This structure is tentative as it does not account for the presence of terminal fucose which appears as 2,3,4-tri-O-methyl-l-fucose in the methylation analyses of both preparations.

The single preparation of the third class (serogroup VI) yielded colitose and 2-acetamido-2-deoxy-D-galactose upon hydrolysis but lacked detectable 6-deoxy-D-manno-heptose, D-mannose, and L-fucose. The results of the sugar analysis indicated that colitose occupies a terminal position (Table 1); more detailed structural studies of this polysaccharide were not attempted.

The methylation analyses also yielded some information regarding the core region of the lipopolysaccharides. d-Glucose, which occurred primarily as 2,3,4,6-tetra-O-methyl-d-glucose, was a component of all preparations and probably derives from the common core as do the heptoses tentatively identified as d-glycero-d-manno- and l-glycero-d-manno-derivatives.

**DISCUSSION**

It was assumed that the 6-deoxyheptose present in lipopolysaccharide of serogroups IA and IVB was identical to the d-manno-derivative recently identified in preparations of serogroup IIA lipopolysaccharide (2, 8). Hexoses and 2-acetamido-2-deoxyhexose were assigned the d-configuration, and fucose was assigned the L-configuration as is generally observed. The alditol acetates of the remaining heptoses had the same retention times as the corresponding derivatives of d-glycero-d-manno- and l-glycero-d-manno-heptose, previously identified as core components of lipopolysaccharides from
other species (6), and it seemed reasonable to assume their presence in preparations from yersiniae. The anomeric natures of the sugars previously encountered in lipopolysaccharides of serogroups IIA and IIB (7, 8) were also assigned the corresponding structures encountered in this study.

The number of oligosaccharide repeating units in the available preparation of yersiniae lipopolysaccharides was small, and thus the O-specific portion of the molecules was much less than that encountered in similar material from other species (13). Accordingly, it was difficult to determine whether a sugar derived from the O-specific side chains or from the basal core of the lipopolysaccharide. Furthermore, small amounts of contaminating polysaccharides may have given significant contributions to results obtained in the sugar and methylation analyses. For these reasons, the structural studies presented here may be less accurate than those generally obtained by methylation analysis, and some discrepancies, mainly of stoichiometrical nature, have been overlooked. It was assumed that preparations which gave qualitatively similar analyses had related structures. These proposed structures, however, must be regarded as tentative.

Nevertheless, accounting for the facts that 3,6-dideoxyhexoses are partially decomposed during acid hydrolysis and that their methylated derivatives are volatile and partially lost in the methylation analysis, there is reasonably good agreement between the postulated structures and the sugar and methylation analyses. The observed immunological cross-reaction between Y. pseudotuberculosis of serogroup IV and Salmonella of serogroup D2 (11) is probably due to the presence of terminal tyvelose residues in their lipopolysaccharides (13). The latter are of the a-configuration in Salomnella which supports the notion of common anomerically.

Serogroups IA and IB, IIA and IIB, and IV A and IVB, respectively, share the common O-factors, 2, 5, and 9 (Table 1). It seems reasonable to assume that this is due to the terminal 3,6-dideoxyhexose residue common to each pair of subgroups. Groups VA and VB also share the common O-factor 10; thus, the absence of ascylose in the latter was not expected. If this deficiency is typical of serogroup VB isolates, then O-factor 10 may reflect the unique (1 → 4) linkage between D-mannose and L-fucose and factors 14 and 15 may represent ascylose and mannose, respectively. The explanation of the antigenic distinction between the paratose-containing serogroups I and III is obvious. Colitose and 2-acetamido-2-deoxy-p-galactose were detected in hydrolysates of lipopolysaccharides of E. coli serogroup 055 (13). The recent observation of an immunological cross-reaction between this lipopolysaccharide and that of yersiniae serogroup VI (14) indicates that the O-specific side chains possess related structures.

The different serogroups of Y. pseudotuberculosis thus express all of the five known 3,6-dideoxyhexoses found in nature. Furthermore, this is the only organism known to produce a 6-deoxyheptose, the p-manno-derivative. The incomplete structural studies reported above indicate that alternative general structures exist for the O-groups. It seems reasonable to assume that only some of the possible combinations of 3,6-dideoxy sugars and general structures have been observed, and it is not improbable that other combinations will be discovered in the future. It is significant that the average number of repeating units in the O-specific side

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**Table 4.** Methyl ethers obtained from the hydrolysates of the fully methylated (A) and the partially hydrolyzed, fully methylated preparations (B) of lipopolysaccharides from Y. pseudotuberculosis serotypes VA, VB, and VI.

<table>
<thead>
<tr>
<th>Sugar*</th>
<th>T₁</th>
<th>T₂</th>
<th>Mol%* for serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>VA</td>
</tr>
<tr>
<td>2,4-Asc</td>
<td>0.29</td>
<td>0.58</td>
<td>6.5</td>
</tr>
<tr>
<td>2,4-Col</td>
<td>0.32</td>
<td>0.99</td>
<td>7.7</td>
</tr>
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<td>2,3,4,6-Man</td>
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<td>1.19</td>
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<td>1.25</td>
<td>1.68</td>
<td>7.7</td>
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<td>2,4,6-Man</td>
<td>2.09</td>
<td>1.90</td>
<td>32.2</td>
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<td>2,3,4,6,7-Hep</td>
<td>2.17</td>
<td>2.73</td>
<td>19.9</td>
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<tr>
<td>2,3,6,7-Hep</td>
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<td>2,3,4,6-Hep</td>
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<td>4.9</td>
<td>10.4</td>
</tr>
<tr>
<td>2,4,6-Hep</td>
<td>12</td>
<td>5.3</td>
<td>0</td>
</tr>
</tbody>
</table>

* The molar percentages are given as in Table 3 except for serotype VI, which is given relative to that of the 2,3,4,6-tetra-O-methyl-glucose derivative, which is assumed to represent all the D-glucose in the sugar analysis.
* See Table 2.
* See Table 3.

**DDHp**

- 1
- 3
- d-Manp(1 → 4)-l-Fucp(1 →

![Fig. 5. Structure of O-specific side chains in polysaccharide in serogroups VA and VB.](http://jb.asm.org/Downloaded from http://jb.asm.org/)
chains are generally small. These values, of course, are only significant for the preparations actually under investigation and may vary between different strains or even between different preparations from the same strain. Perhaps alternative methods of purification would yield preparations containing a greater number of O-groups.

It is not known whether yersiniae discriminate between L-glycero- and D-glycero-D-manno-heptoses in the biosynthesis of the core. The fact that two 2,3,4,6,7-penta-O-methylheptoses were detected in some methylation analyses (serogroups IB and VA) indicates that such discrimination does not exist. The penta-O-methyl derivative with the higher retention time is probably L-glycero-D-manno-heptose, previously encountered in methylation analyses of Salmonella lipopolysaccharide (6). Most of the preparations gave the same heptose derivatives in methylation analyses, suggesting that the core structures are of the same or similar configuration. A poor agreement between the relative percentages of heptoses observed in sugar and methylation analyses was also observed in earlier studies of lipopolysaccharides from Salmonella and may have been due to phosphorylation of some heptose residues.

ACKNOWLEDGMENTS

The skilled technical assistance of Jana Cederstrand and Prudence Hall is acknowledged.

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LITERATURE CITED