Characterization of structural elements of the lipopolysaccharides of \textit{Pasteurella multocida}

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Characterization of structural elements of the lipopolysaccharides of Pasteurella multocida

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W. Erler, H. Feist, K.D. Flossmann, B. Jacob and A. Pilarski

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Summary - The main fraction of oligosaccharides obtained following acetic acid hydrolysis of the lipopolysaccharides (LPS) of strain PM contained: terminal-bound glucose and L-glycero-D-mannoheptose, 1,2-linked, 1,3,4-linked, and 1,3,4,6-linked heptose. The oligosaccharides of strain 1297 S contained, in addition, terminal-linked galactose, 1,4-linked or 1,6-linked glucose and 1,4-linked N-acetylglucosamine. The molar ratios of these elements reflect the heterogeneity of the preparations. The molecular weights determined underline the R character of the LPS. The known mannan and another polysaccharide with galactose and glucosamine were identified in the subsidiary fractions obtained following acetic acid hydrolysis.

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KEY to the Tables and Figures

aus  from
Gal  galactose
GalN galactosamine
Glc  glucose
GlcN glu
GAZ total amino sugars (TAS)
D-Hep D-glycero-D-mannoheptose
L-Hep L-glycero-D-mannoheptose
KDO 2-keto-3-deoxyoctonate, or (=)
     2-keto-3-deoxyoctonic acid
LPS  lipopolysaccharides
Molekulargewicht molecular weight
PM0P dephosphorylated PM
RKH reducing carbohydrates (RCH)
Summe Total (for ...)
wie like
In preceding communications (Erler et al., 1977; 1981), we have described certain general properties of lipopolysaccharides (LPS) obtained from various Pasteurella multocida strains, and demonstrated that these LPS possess R character in chemical terms, and—in correspondence to the qualitative occurrence of the monosaccharides glucose (Glc), galactose (Gal), glucosamine (GlcN), Galactosamine (GalN) and L- and D-glycero-D-manno-heptose (L-Hep and D-Hep, respectively)—can be assigned to at least four chemotypes. We have continued our investigation of the P. multocida LPS in order to identify the structural elements of the polysaccharide moiety of the LPS. The present communications reports the results obtained in the polysaccharides of the LPS of strains PM and 1297 S.

Material and Methods

The P. multocida strain PM was made available to us by the National Institute of Animal Health, Tokyo [Japan]; strain 1297 S had been isolated in the Department of Microbiology of our Institute from a pig suffering from enzootic pneumonia. In correspondence to the monosaccharides contained in the LPS, strain PM belongs to chemotype I (Glc, GlcN, L-Hep), and strain 1297 S belongs to chemotype II (Glc, Gal, GlcN, L-Hep) (Erler et al., 1977).

Cultivation of the bacteria, their extraction with phenolic water, purification of the LPS as well as the analytical procedures for determining the reducing carbohydrates (RCH), the total amino sugars (TAS), glucose (Glc), and the ketodeoxyoctonic acid (KDO), the permethylation of the polysaccharides, and the gas chromatographic and mass-spectrometric identifications of the monosaccharides and of the partially methylated monosaccharides have already been described (Erler et al., 1977; 1980; 1981). Hydrolysis
of the LPS with diluted acetic acid, and the separation of the oligo-
saccharides on Sephadex G-50 or Sephadex G-25, respectively, were per-
formed as described by Mueller-Seitz et al. (1968).

The contents of acetyl groups were determined by gas chromatographic
means on PEG 9000 (2 m; 60° isothermal) as methyl acetate following treat-
ment of the oligosaccharides with 5% methanolic hydrochloric acid (one
hour, 100°C).

Determination of the molecular weights was performed on Sephadex G-25
superfine (1.5 x 2.5 cm; 1-mL fractions; eluting agent: 0.2 M Nh4HCO3 sol-
ution). The column was calibrated with glucose, maltose, raffinose, and a
partial hydrolysate of Dextran 10 (0.5 N H2SO4, 2.5 hours).

The oxidation with periodate was performed as follows: 5 mg oligo-
saccharide were dissolved in 5 mL water, whereupon 5 mL of a 25 mM sodium
periodate solution were added; the mixture was stored for 96 hours in a
refrigerator, mixed with 10 µg glycol, and then reduced with NaBH4. Follow-
ing discontinuation of reduction, known quantities of arabinose were added
followed by quantitative determinations. The borate was removed, the
mixture subjected to hydrolysis, and the samples were processed for gas-
chromatography of the alditolacetates.

Results

Hydrolysis of the LPS with diluted acetic acid

Figure 1 illustrates the elution profile of the oligosaccharides follow-
ing hydrolysis of the LPS of strain 1297 S with diluted acetic acid. In
the case of strain PM, Fraction IV represents the main fraction. These re-
sults underline the finding made already previously (Erler et al., 1980),
Figure 1 - Elution of the oligosaccharides on Sephadex G-50 following hydrolysis of LPS strain 1297 S with acetic acid

viz. that the LPS exhibit an unequivocal R character. In the case of S lipopolysaccharides, hydrolysis with diluted glacial acetic acid yields an elution profile in which Fraction I—corresponding to the exclusion volume of Sephadex G-50—represents the main fraction.

The recovery rates during fractionation (related to the polysaccharide added) amounted to between 87 and 92 per cent following acetic acid hydrolysis, demonstrating that no substance had escaped identification.

On re-chromatography on Sephadex G-25, the main fractions behaved in homogenous fashion.

Characterization of the subsidiary fractions:

Fractions I, II and III of strain PM and Fractions I, II and IV of strain 1297 S were counted with the subsidiary fractions. Only small quantities of these fractions were available to us, so that we were able to provide only rough characterizations.

In Fractions I of both strains, we were able to identify mannose in addition to small quantities of Glc using gaschromatographic means. The
positive precipitation of the two fractions in agar gel with Concanavalin A as well as the results of the permethylation and the determination of the partially methylated alditol acetates in Fraction I of strain PM correspond to the results obtained in the mannan of the pasteurellas (Erler et al., 1981), so that we hold the view that Fraction I of these two strains consists chiefly of that polysaccharide.

Table 1 summarizes analytical data obtained in Fractions II of the two strains. In essence, these data indicate:

a concentration of the amino sugars takes place; and
galactose represents the main constituent of the neutral sugars.

In Fraction III of strain PM, we found 48% RCH, 7.5% TAS, 17% Glc, and 0.8% KDO, and, by gaschromatographic means, mainly Glc in addition to traces of mannose, Gal and L-Hep as neutral sugars.
Fraction IV of strain 1297 S contained neither amino sugars nor neutral sugars. In that fraction, we found 17% KDO.

Characterization of the main fractions

In correspondence to the results of acetic acid hydrolysis of the LPS and of gel filtration of the polysaccharide moiety separated from lipid A on Sephadex G-50, Fraction IV of strain PM and Fraction III of strain 1297 S must be counted with the main fractions. Table 2 gives the data obtained in general analytical investigation.

In addition, we have investigated in these fractions the occurrence of further Ninhydrin-positive compounds using an amino-acid analyser; the occurrence of ribitol and glycerol using gaschromatographic means; and that of pyruvic acid, succinic acid and uronic acid, which have been described as constituents of certain lipopolysaccharides. All these tests were negative.

Analysis of the linkages of the individual monosaccharides was aimed at providing further insights into the structures of these polysaccharides.

For the purpose of these analyses, we have made use of the method described by the working group of Bjoerndal and Lindberg (Bjoerndal et al., 1967; 1970; Loenngren and Svensson, 1974) for investigating bacterial polysaccharides, i.e. the method of permethylation by means of methylsulfinyl carb-anion and methyl iodide, and identification of the partially methylated alditolacetates following hydrolysis and derivation with the aid of gas-chromatography or combined gaschromatography and mass-spectrometry, respectively. For better identification of the substances, we have used NaBD₄ instead of NaBH₄ in the preparation of the alditols in a part of the hydrolysates.

Fraction PM IV - Among the fractions selected, that polysaccharide exhibits the most simple composition, since it contains only Glc and L-Hep
as bound monosaccharides. To be sure, we found in that fraction 1.9% P (Table 2), so that we must assume that phosphoric acid [phosphate] is bound in this polysaccharide. For that reason, we had to perform dephosphorylation with the aid of hydrofluoric acid (Haemmerling et al., 1973).

We have compared the P-containing polysaccharide with the dephosphorylated one in the methylating reaction. The gaschromatograms of the partially methylated alditolacetates are presented as Figure 2.

In the case of the P-containing polysaccharide (PM), we obtained six peaks in the gaschromatogram; in that of the dephosphorylated one (PM₀P), the number of peaks was reduced to five. Table 3 lists the retention times, the area percentages determined, the primary fragments obtained following reduction with NaBH₄(H) and NaBD₄(D), respectively, in the mass spectra, and the methylated compounds identified on the basis of these spectra.

Summation of the quantities of monosaccharides determined in the PM₀P yielded values showing very good agreement with the values obtained following total hydrolysis. Conversion of the absolute values given in Table 2 for Glc and L-Hep to relative percentage values yields the following pattern:

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Methylation</th>
<th>Total Hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glc</td>
<td>37%</td>
<td>37.4%</td>
</tr>
<tr>
<td>L-Hep</td>
<td>63%</td>
<td>62.6%</td>
</tr>
</tbody>
</table>

**Fraction 1297 S III** - Despite the low P contents, we compared also in this fraction the untreated oligosaccharide with the hydrofluoric acid-treated one using the methylating procedure. We obtained no differences worth mentioning, so that we have used the untreated oligosaccharide exclusively in our work. A gaschromatogram of the partially methylated alditolacetates is presented as Figure 3; the analytical data are summarized in Table 4.
Figure 2 - Gaschromatograms of the partially methylated alditolacetates of Fraction PM IV. ----, containing P; ----, dephosphorylated.

Table 3 - Partially methylated alditolacetates of Fraction PM IV

<table>
<thead>
<tr>
<th>No.</th>
<th>Area percents</th>
<th>Primary fragments</th>
<th>Alditolacetate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R Me,Glc</td>
<td>PM</td>
<td>PMoP</td>
</tr>
<tr>
<td>1</td>
<td>1.0</td>
<td>41</td>
<td>37</td>
</tr>
<tr>
<td>2</td>
<td>2.1</td>
<td>29</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>4.2</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td>4</td>
<td>5.4</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>6.2</td>
<td>5</td>
<td>--</td>
</tr>
<tr>
<td>6</td>
<td>10.1</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>10.1</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>10.1</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>
Figure 3 - Gaschromatogram of the partially methylated alditolacetates of Fraction 1297 S III.

Compared to Fraction PM IV, this oligosaccharide of strain 1297 S exhibits two additional peaks [in the gaschromatogram] at $R_{Me_4Glc} = 1.23$ and 2.5, respectively. On the basis of the retention values and that of the mass spectrum, which is identical with that of 2,3,4,6-Me$_4$Glc, the former peak could be identified as corresponding terminal-linked galactose. In correspondence with the values available in literature, we must give consideration to 2,3,6- or 2,3,4-Me$_3$Glc in the case of the second peak. The mass spectra do not permit unequivocal assignment. In the combination of the primary fragments, these two alditolacetates differ in the occurrence of masses 45 and 189, respectively, and 205. We have been able to identify all three primary fragments. On the basis of the primary fragments determined, we are permitted to conclude that this particular peak represents a mixture of the two alditolacetates; we have been unable to determine their ratio.
Table 4 - Partially methylated alditolacetates of Fraction 1297 S III

<table>
<thead>
<tr>
<th>No.</th>
<th>RMe₆Glc</th>
<th>%</th>
<th>Primary fragments</th>
<th>Alditolacetates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
<td>11</td>
<td>wie Peak 1 PM IV</td>
<td>2.3.4.6 Me₆Glc</td>
</tr>
<tr>
<td>2</td>
<td>1.35</td>
<td>18</td>
<td>wie Peak 1 PM IV</td>
<td>2.3.4.6 Me₆Gal</td>
</tr>
<tr>
<td>3</td>
<td>2.85</td>
<td>19</td>
<td>wie Peak 2 PM IV</td>
<td>2.3.4.6 Me₆L-Hep</td>
</tr>
<tr>
<td>4</td>
<td>2.5</td>
<td>20</td>
<td>R: 45, 117, 161, 189, 205, 233</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4.1</td>
<td>20</td>
<td>D: 45, 118, 162, 189, 206, 233</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>5.5</td>
<td>5</td>
<td>wie Peak 3 PM IV</td>
<td>2.3.4 Me₆Glc</td>
</tr>
<tr>
<td>7</td>
<td>10.0</td>
<td>7</td>
<td>wie Peak 6 PM IV</td>
<td>2.6.7 Me₆L-Hep</td>
</tr>
</tbody>
</table>

Table 5 - Comparison of the analytical values obtained (1297 S III)

<table>
<thead>
<tr>
<th>Total hydrolysis (%)</th>
<th>Methylation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glc</td>
<td>19.4 ± 32.4</td>
</tr>
<tr>
<td>Gal</td>
<td>8.7 ± 14.5</td>
</tr>
<tr>
<td>L-Hep</td>
<td>31.8 ± 58.1</td>
</tr>
</tbody>
</table>

Summation of the monosaccharides determined and comparison of the sum totals with the values of total hydrolysis converted to relative percentages (Table 2) yields the numerical data presented in Table 5, which agree to a satisfactory extent. The fraction furthermore contains 3.4 per cent GlcN. In order to determine the type of linkage of GlcN, we proceeded from the hydrolysate of the methylated oligosaccharides. Following conversion of the monosaccharides into their alditols, the solution was treated with a cation exchanger for separation of the cations, with the amino sugars also being bound to the exchanger. The amino sugars were removed with the aid of hydrochloric acid, converted into their alditolacetates after neutralization, and chromatographed on ECNSSM at 190°C in a 1-m column. In the gaschromatogram, we found in each instance a peak after approximately 13 minutes. The mass-spectrometric investigation (Figure 4) of the substances reduced with
Figure 4 - Mass spectra of the amino sugar fraction

Table 6 - Contents of Man and L-Hep (in %, related to total L-Hep) determined following periodate oxidation compared to the values estimated on methylation

<table>
<thead>
<tr>
<th></th>
<th>Periodate oxidn.</th>
<th>Methylation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PM XIV</td>
<td>1297 S III</td>
</tr>
<tr>
<td>Man</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>L-Hep</td>
<td>8</td>
<td>14</td>
</tr>
</tbody>
</table>

NaBH₄ as well as with NaBD₄ [respectively] unequivocally indicated the presence of 1,4,5-tri-O-acetyl-3,6-di-O-methyl-N-acetyl-N-methyl-glucosaminitol, and, thus, the presence of 1,4-linked glucosamine in the oligosaccharide.
Results of oxidation with periodate

Oxidation with periodate represents a procedure generally used in the elucidation of polysaccharide structures. We have employed this particular method in order to confirm the results of the methylating procedure. In all main fractions we identified 1,3,4,6-linked L-glycero-D-mannoheptose, which is resistant to periodate. Following oxidation with periodate, a part of the L-Hep would thus have to be found in the hydrolysate of all oligosaccharides.

We have identified 1,3,4-linked L-Hep in the main fractions of strains PM and 1297 S. Periodate oxidation resulted in cleavage of the link between C\textsubscript{6} and C\textsubscript{7} of that heptose, so that mannose could be demonstrated following reduction and hydrolysis.

The results of oxidation with periodate revealed that L-Hep and mannose can be identified in each one of the oligosaccharides. The quantitative assessment (Table 6) indicates that the results of periodate oxidation are correlated with the results obtained using the methylating procedure.

Discussion

The eluting profile of the oligosaccharides following hydrolysis of the LPS with acetic acid (Figure 1) and the molecular weights determined in the main fraction (Table 2) underline the finding reported already in a previous paper (Erler et al., 1980) viz. that the LPS of *P. multocida* have R character.

No exact data can be reported regarding the subsidiary fractions obtained only in small quantities. Fraction I contained considerable quantities of the mannan found in all *P. multocida* strains hitherto investigated; the
structure of that mannan has been elucidated (Erler et al., 1980b [sic! 1980?]). So far we have been unable to establish whether the glucose identified is derived from a S-LPS perhaps existing or whether we are dealing with a polysaccharide differing from LPS and from the mannan, i.e. a glucan.

Due to their high Gal and amino-sugar contents, the compositions of Fractions II of the two strains indicate that—in addition to the LPS and the mannan—still other polysaccharides are bound in the cell walls of the pasteurelles, which polysaccharides are present only in small quantities or are not completely removed using the phenolic water method.

KDO and the heptose were present to an increased extent in Fraction III of strain PM. However, the content of glucose was predominant; that content was higher than the ones determined in the main fractions. Also in this instance, we were unable to establish whether Glc is attached to the R-LPS.

In the main fractions, we found 49 and 61 per cent, respectively, reducing carbohydrates (Table 2, column 1). These values are somewhat too low for pure polysaccharides. Comparisons with data available in literature, however, indicate that the values determined by us in these R-polysaccharides are in agreement with those reported by other authors. For instance, Prehm et al. (1975; 1976a; 1976b) found in various R-polysaccharides of Escherichia coli mutants approximately 40 per cent to maximally 80 per cent, and, as a rule, about 60 per cent coonosaccharides; Sutherland et al. (1965) in oligosaccharides of Salmonella R-mutants between 50 and 59 per cent monosaccharides; and Schmidt et al. (1970) in the oligosaccharide of a Mre-polysaccharide of Citrobacter 47.5 per cent monosaccharides.
Our findings can be explained on the basis of the fact that hydrolysis with diluted acetic acid entails an attack at the ketosidic-bound KDO residues, while one KDO molecule remains bound to the core polysaccharide (Prehm et al., 1975; Drewy et al., 1975); however, that state cannot be demonstrated due to the acid instability or due to the linkage of the heptose by way of the C$_5$-OH group of KDO, respectively. Due to that linkage, formyl-pyruvic acid—i.e. the chromogen for the detection and identification of KDO with the aid of thio-barbituric acid (Ghalambar et al., 1966; Drewy et al., 1975)—is not being formed during oxidation with periodate. Depending on the R-character, the share of 1 mole KDO in the oligosaccharide amounts to about 10 to 20 per cent.

The quantity of the amino sugars determined (Table 2, column 2), which in qualitative terms are related exclusively to GlcN, is interesting. The 0.5 per cent of GlcN found in the main fraction of strain PM must be attributed, without doubt, to contamination, so that the GlcN determined in the LPS is derived exclusively from lipid A. Since we, following hydrolysis with acetic acid of the LPS of strains P 8 and D 33, which also belong to chemotype I (Erler et al., 1977), were able to demonstrate in the main fraction obtained on Sephadex separation no or only 0.16 per cent GlcN, respectively, we would be permitted to conclude that the polysaccharides of the strains of chemotype I do not contain bound GlcN, and that the GlcN found is derived from lipid A.

Columns 2 to 5 of Table 2 give the quantities of the monosaccharides determined. The qualitative occurrences of heptose, of Glc, and of Gal correspond to the contents found in the chemotypes of the LPS. The sums of the individual monosaccharides are well correlated with the amounts of reducing carbohydrates determined, as is demonstrated by comparing columns 1 and 6.
Noticeable quantities of phosphorus could be detected only in the fraction of strain PM. We regard the P contents of the other fractions as being due to contamination.

The results obtained using the methylating procedure indicate that the two polysaccharides contain the terminal-linked Glc (Glc 1-) and L-Hep (L-Hep 1-) and the branched L-Hep (-3,4,6-L-Hep 1-).

Terminal-linked Glc has been demonstrated also in the core polysaccharides *coli* R₁ (Kiss et al., 1978), *coli* R₃ (Mayer, 1972), *coli* B (Prehm et al., 1975), *Pseudomonas* (Drewy et al., 1975), and in *Yersinia pestis* (Bordet et al., 1977), while terminal-bound L-Hep is contained in almost all core polysaccharides with elucidated structures. With respect to these two structural elements, our results obtained in *P. multocida* are in line with the known core structures.

However, a heptose as branched as the 1,3,4,6-linked L-Hep identified by us has hitherto not been described. Our result is supported by the findings obtained on oxidation with periodate (Table 6). In both polysaccharides, we have been able to demonstrate quantities of periodate-resistant L-Hep corresponding to the quantity of 1,3,4,6-linked heptose found using the methylating procedure. To be sure, the 1,3,7-linked and 1,3,6-linked heptoses present in the cores of Enterobacteriaceae are also periodate-resistant; the retention times of these heptoses have been reported to be $R_{\text{Me}_4\text{Glc}}$ = 13.5 to 13.8 (Haemmerling et al., 1970; Prehm et al., 1975). Apart from the retention times, they differ clearly with respect to their mass spectra. Our mass spectra exhibit fragments 45, 117, and 377, and 45, 118, and 377, respectively, which permit no other conclusion. Retention behavior and mass spectra are in agreement also with those reported by Radziejewska-Lebrecht et al. (1979).
The 1,2-linked L-Hep was demonstrated by us in both oligosaccharides. This type of linking—that of a 'chain heptose'—also has hitherto not yet been identified. The one demonstrated in all basal oligosaccharides of the Enterobacteriaceae is the 1,3-linked heptose. Since the retention times ($R_{Me_4Glc} = 4.4$ for 1,3-L-Hep; Prehm et al., 1976a) are almost identical, our results are supported by the mass-spectrometric evidence and by the data reported by Radziejewska-Lebrecht et al. (1979).

The absence of Peak No. 5 in the dephosphorylated polysaccharide of strain PM and the doubling of the quantity [area] of Peak No. 3 permit us to conclude that the phosphoric acid is located at C$_3$ of the 1,2-glycosidic-bound heptose.

As single-branched L-Hep, we have demonstrated the 1,3,4-linked heptose in the oligosaccharides. That heptose has been identified also in Y. pestis, in addition to 1,3,7-linked heptose (Bordet et al., 1977); this particular heptose does not occur in other Enterobacteriaceae. Using a temperature program, Bordet has reported a retention time of $R_{Me_4Glc} = 4.88$, while our fraction, using the isothermal procedure, is characterized by $R_{Me_4Glc} = 5.4$. The combinations of the primary fragments correspond, and are also identical with those described by Radziejewska-Lebrecht et al. (1979).

The polysaccharide of strain 1297 S contains, in addition, terminal Gal (Gal 1-), 1,4-linked and 1,6-linked glucose (-4 Glc 1- and -6 Glc 1-), respectively, and 1,4-linked N-acetylglucosamine (-4 GlcNAc 1-). Terminal Gal has been described frequently in core polysaccharides, and also in Y. pestis (Bordet et al., 1977). 1,4-Linked Glc has been found in Y. pestis, and 1,6-linked Glc in Pseudomonas aeruginosa. As already outlined further above, the mass-spectrometric investigation permitted no unequivocal assignment, so that we are forced to assume that both types of linkage are found in these polysaccharides.
With respect to the 1,4-linked glucosamine, its occurrence in *P. multocida* is hitherto unique. Terminal glucosamine is a component of many cores; however, glucosamine linked in series has as yet not been described in core polysaccharides. Only *Pseudomonas aeruginosa* contains GalN at one branching site. Also in that instance, mass-spectrometric investigation ultimately settled the identification.

In Fraction PM IV, we determined the molar ratio of L-Hep:Glc (from the total hydrolysate) to amount to about 3:1.8. The results obtained using the methylating procedure confirm that ratio in summary terms, but indicate clearly that four differently linked heptoses are present in the polysaccharides, having the following relationships (rounded molar values):

<table>
<thead>
<tr>
<th>L-Hep</th>
<th>Gal</th>
<th>Glc</th>
<th>4 Glc and 6-Glc</th>
<th>1-Hep</th>
<th>2-Hep</th>
<th>3.4 L-Hep</th>
<th>3.4.6 L-Hep</th>
<th>GlcNAc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>(1.0 Mole)</td>
<td>(0.0 Mole)</td>
<td>(0.0 Mole)</td>
<td>(0.3 Mole)</td>
<td>(0.3 Mole)</td>
<td>(0.3 Mole)</td>
<td>(0.3 Mole)</td>
<td>(0.3 Mole)</td>
</tr>
</tbody>
</table>

That finding demonstrates in unequivocal terms that the preparations are homogenous.

The situation is similar in the case of Fraction 1297 S III. In the total hydrolysate, we determined a Gal:Glc:L-Hep molar ratio of about 1:2:3. The following distribution is derived from the quantities of partially methylated alditol acetates identified:

<table>
<thead>
<tr>
<th>Gal</th>
<th>Glc</th>
<th>4 Glc and 6-Glc</th>
<th>1-Hep</th>
<th>2-Hep</th>
<th>3.4 L-Hep</th>
<th>3.4.6 L-Hep</th>
<th>GlcNAc</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6</td>
<td>0.6</td>
<td>1.05</td>
<td>0.8</td>
<td>1.0</td>
<td>1.06</td>
<td>1.05</td>
<td>0.4</td>
</tr>
<tr>
<td>(0.6 Mole)</td>
<td>(0.6 Mole)</td>
<td>(1.05 Mole)</td>
<td>(1.0 Mole)</td>
<td>(1.0 Mole)</td>
<td>(1.06 Mole)</td>
<td>(1.05 Mole)</td>
<td>(0.4 Mole)</td>
</tr>
</tbody>
</table>
According to that summation, 0.3 to 0.5 mole glucose is missing. In addition to the main peaks, we obtained in the gaschromatograms several very low peaks, which we were unable to identify. They may be associated with the glucose.

With respect to the contents of branched heptoses and of the glucosamine determined, the same is valid as mentioned in connection with the oligosaccharide of strain [Fraction] PM IV. We hold the view that our substances represent mixtures despite the homogenous peak shape obtained following chromatography on Sephadex. This heterogeneity of the LPS of P. multocida is indicated already by the identification of heptoses in the higher molecular fractions obtained on separation on Sephadex G-50. For that reason, we have not carried out attempts to outline the structures of these oligosaccharides.

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Bibliography


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Translation of foreign-language titles

1. Characterization of the lipopolysaccharides of several *Pasteurella multocida* strains.

2. Further characterization of the lipopolysaccharides obtained from *Pasteurella multocida*.

3. Structural investigation of a mannan obtained from *Pasteurella multocida*.

4. Reactivity and differentiation of the complete enterobacterial R-basal with the aid of concanavaline.