Studies on the antigenic S-type lipopolysaccharides of *Brucella abortus* strains 7 and Mustapha

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Antigenic phenol phase S-type lipopolysaccharides (LPS) isolated from *Brucella abortus* (B. abortus) strains 7 and Mustapha were observed to have $^{13}$C n.m.r. spectra which were almost identical to the one reported for the *Brucella abortus* 1119-3. The glycosyl content of the lipid A obtained from the LPS of strain 7 was found to be 2-acetamido-2-deoxyglucose only while strain Mustapha was found to contain both 2-acetamido-2-deoxyglucose and 2-acetamido-2-deoxygalactose. The fatty acid present in the lipid A of both strains was mainly n-hexadecanoic acid. Octadecanoic acid, 3-hexadecenoic acid as well as small quantities of 3-hydroxydodecanoic acid were also identified. This contrasts with the earlier reports of the absence of 3-OH-14:0 in the LPS of *Brucella abortus*.

Keywords: *Brucella abortus*; lipopolysaccharide; n.m.r. spectroscopy; fatty acids

Introduction

*Brucella abortus* is the main cause of contagious abortion in cattle. The disease brucellosis was first reported in Nigeria in 1928 and it has been shown to be a problem to all breeds of cattle in that country. The overwhelming majority of brucellosis outbreaks in Nigeria are caused by biotypes 1, 2 and 3. The economic importance of brucellosis calls for extensive study of the causative organism. Earlier literature contains some reports on the identification of glucose, galactose and mannose in the LPS of *Brucella abortus*. However Carroff et al. carried out extensive studies on the antigenic S-type LPS of *B. abortus* 1119-3. This led to the identification of the O-chain polysaccharide of *B. abortus* S-type lipopolysaccharide as a linear homopolymer of 1,2-linked 4,6-dideoxy-4-formamido-3-0-mannopyranosyl units. Kreutzer et al. observed that hexadecanoic acid and octadecanoic acid were the major fatty acid constituents in the lipid and also noted the absence of 3-hydroxytetradecanoic acid, a common marker of enteric LPS. However Caroff et al. reported the presence of 3-hydroxy tetradecanoic acid in the LPS of *B. abortus*.

In this report, we present the results of the chemical studies on the LPS from the cell wall of *B. abortus* biotype 1, strains 7 and Mustapha which among others have been implicated in brucellosis in Nigeria.

Experimental

All the reagents used in this report were of analytical grade. The following strains of *B. abortus*: biotype 1 strain 7 and biotype 1 strain Mustapha typed at the Ministry of Agriculture and Veterinary Laboratory, New Haw, Weybridge, Surrey, England, were obtained from the collection of the National Veterinary Research Institute (NVRI) Vom. Strain 7 was isolated from the South Devon breed 27 at the Livestock Investment Breeding Centre in Kano while the strain Mustapha was isolated from Alhaji Mustapha's herd, a local milk collection centre in Maiduguri.

Culturing conditions

Each strain was supplied as a lyophilized culture and was grown on potato dextrose agar under CO$_2$ for 48 h at 37°C. The bacterial growth was harvested with sterile 1% saline. The cells were then centrifuged and killed with acetone to obtain the acetone dried cells which gave an average yield of 17 mg from 100 mg of wet cells.

Extraction of the crude LPS

The LPS was extracted by the hot water-phenol method. Twenty g dry weight of each *B. abortus* strain gave an average yield of 1.7 g of the crude aqueous phase LPS and 2.0 g of the crude phenol-phase LPS (PPLPS). The latter was purified according to the method of Moreno et al. and a yield of 32 mg was obtained from 100 mg of the crude.

Isolation of the polysaccharide (PS) and lipid A from the LPS

Delipidation was by mild acid hydrolysis with 0.1 M acetic acid as described in another report. Fifty mg of the LPS gave the following yield of PS: 22 mg of strain 7 and 23 mg of strain Mustapha.
\[ ^{13}C \text{n.m.r.} \]

To 20 mg of each PS was added 0.4 ml of 99% D\textsubscript{2}O. The spectra were recorded on a GX-100 Jeol spectrometer at 70°C with full proton decoupling. Chemical shifts are reported using dioxane \((^{13}C \delta = 67.4 \text{ppm})\) as internal reference for strain 7 and acetone as internal reference \((^{13}C \delta = 31.04 \text{ppm})\) for strain Mustapha.

**Sugar analysis of lipid A\textsuperscript{12,13}**

The method of Stoneker\textsuperscript{13} was employed with some modifications\textsuperscript{12}. The alditol acetates were analysed on a Hewlett Packard 5830A instrument fitted with a flame ionization detector and an SE-54 fused capillary column. Both injector and detector temperatures were 270°C while the column temperature was 185°C held for 8 min and increased to 230°C at 5°C/min. In order to determine the percentage of the backbone sugars present in the lipid, the sugar analysis was repeated using mannose as internal standard.

**Fatty acid analysis**

This was carried out as previously described\textsuperscript{12}.

**Results and discussion**

The \(^{13}C \text{n.m.r.} \) data of the phenol O-polysaccharides of the two strains are recorded in Table 1. The decoupled spectra of the strain 7 showed signals at 101.32, 77.93, 68.56, 17.79 and 166.76 ppm and are assigned as for strain 7.

Table 1 \(^{13}C \text{n.m.r.} \) shifts of the native O-chain from the phenol-phase LPS of the B. abortus strain 7 (S-7) and strain Mustapha (S-M)

<table>
<thead>
<tr>
<th>Carbon atom</th>
<th>Shifts O-PS (ppm)</th>
<th>((\delta^c-\delta) \text{ ppm} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(S-7)</td>
<td>(S-M)</td>
</tr>
<tr>
<td>C-1</td>
<td>101.32</td>
<td>101.43</td>
</tr>
<tr>
<td>C-2</td>
<td>77.93</td>
<td>77.98</td>
</tr>
<tr>
<td>C-3</td>
<td>69.01</td>
<td>69.11</td>
</tr>
<tr>
<td>C-4</td>
<td>52.93</td>
<td>52.98</td>
</tr>
<tr>
<td>C-5</td>
<td>68.56</td>
<td>68.53</td>
</tr>
<tr>
<td>C-6</td>
<td>17.79</td>
<td>17.79</td>
</tr>
<tr>
<td>NHCHO</td>
<td>165.60</td>
<td>166.76</td>
</tr>
</tbody>
</table>

\(\delta^c\) = chemical shift values of the carbon atoms in the native O-chain of S-7 and S-M

**Fatty acid analysis**

The \(^{13}C \text{n.m.r.} \) data of the phenol O-polysaccharides of the two strains are recorded in Table 1. The decoupled spectra of the strain 7 showed signals at 101.43, 77.98, 69.11, 52.98, 68.53, 17.79 and 166.76 ppm and are assigned as for strain 7. Table 1 also showed the values of \((\delta^c-\delta)\) where \(\delta^c\) are the chemical shift values reported for the 1,2-linked 4,6-dideoxy-4-formamido-\(\alpha\)-mannopyranosyl residue present in the LPS of the B. abortus 1119-3\textsuperscript{8}. The single signal present in the anomeric region indicates that the PS is a homopolymer.

### Table 1 Characterization of the fatty acids present in the phenol phase LPS and the isolated lipid A of B. abortus strains 7 (S-7) and Mustapha (S-M)

<table>
<thead>
<tr>
<th>Fatty acid methyl ester</th>
<th>G.I.C. relative retention time ((t_R))\textsuperscript{a}</th>
<th>% of fatty acids</th>
<th>Mass spectrometry</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LPS</td>
<td>Lipid A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(S-7)</td>
<td>(S-M)</td>
</tr>
<tr>
<td>14:0</td>
<td>1.00</td>
<td>94</td>
<td>3</td>
</tr>
<tr>
<td>16:0</td>
<td>1.41</td>
<td>64</td>
<td>73</td>
</tr>
<tr>
<td>18:0</td>
<td>1.78</td>
<td>23</td>
<td>18</td>
</tr>
<tr>
<td>3-OH-12:0</td>
<td>0.88</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>3-OH-14:0</td>
<td>1.33</td>
<td>13</td>
<td>6</td>
</tr>
</tbody>
</table>

\(\text{a}\) Relative retention times are based on tetradecanoic acid methyl ester (14:0; \(t_R = 1.00\))

\(\text{b}\) Signals were very weak on g.c.-m.s

\(\text{c}\) Although strong signals were observed on the g.i.c. the response under the operating conditions of the g.c.-m.s. was rather weak

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In view of these earlier reports, a minimum of about 10 determinations was carried out on the LPS of each strain and this fatty acid was found present in all the determinations. Since the final proof of its presence rested on mass spectral analysis, the samples were injected on the g.c.-m.s. However, at the operating conditions of the instrument, the signal corresponding to 3-hydroxytetradecanoic acid on the g.l.c. was very weak on the g.c.-m.s. Because of this poor response, it was not possible to obtain the mass spectra of the fatty acid. The identification therefore rested on the results from the g.l.c. and a typical chromatogram is shown in Figure 1. It is quite possible that the use of capillary g.l.c. in this analysis has made this identification possible.

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