ANALYSIS OF THE 3-DEOXY-D-MANNO-2-OCTULOSONIC ACID REGION IN A LIPOPOLYSACCHARIDE ISOLATED FROM *COXIELLA BURNETII* STRAIN NINE MILE IN PHASE II

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Summary. – Structural analysis of the 3-deoxy-D-manno-2-octulosonic acid (Kdo) region in a lipopolysaccharide (LPS) II isolated from *Coxiella burnetii* strain Nine Mile in avirulent phase II revealed the presence of three variously linked Kdo residues. The lipid A proximal Kdo is substituted at C-4 by a Kdo-(2–>4)-Kdo disaccharide and this structural arrangement of the Kdo residues is similar to that of enterobacterial LPSs.

Key words: *Coxiella burnetii;* strain Nine Mile; phase II; lipopolysaccharide; structure; Kdo region

In the structural studies of LPS II isolated from *C. burnetii* strain Nine Mile in avirulent phase II, Schramek and Mayer (1982) have reported on the presence of "Kdo-like substance" as a constituent sugar of lipid A proximal region. Their finding was based solely on the detection of the substance which was positive in a thiobarbituric acid (TBA)-periodate spray assay (Anderson, 1966) and had a different mobility with respect to that of the authentic Kdo on high-voltage paper electrophoresis. Since that time no further studies have been undertaken in this direction and the term "Kdo-like substance" has been used in subsequent papers (Amano *et al.*, 1987; Mayer *et al.*, 1988) dealing with the structure of *C. burnetii* LPSs. There is no doubt, however, that the above finding is highly ambiguous unless supported by the relevant methods of chemical structure analysis. In our recent paper (Toman *et al.*, 1993), the presence of Kdo in LPS II was unequivocally established by gas chromatography-mass spectrometry (GC-MS). No other derivative or analogue of Kdo could be found by the method applied. The quantitative determination of Kdo by the TBA assay indicated the presence of three Kdo residues (Toman, 1992) in LPS II. Therefore, it was of interest to prove this result by the more reliable methods of chemical structure analysis and to establish the structural arrangement of Kdo residues in LPS II. The results are reported herein.

The LPS II (5 mg) was solubilized in methyl sulfoxide (0.7 ml) and methylated (Ciucanu and Kerek, 1984) with methyl iodide (0.5 ml) in the presence of NaOH (15 mg) for 2 hrs at room temperature. The methylated LPS II was purified on Sep-Pak C_{18} cartridge and carboxyl-reduced with $NaBD_4$ (15 mg) in methanol-water (2 ml, 1:1) at 7 °C for 18 hrs. After addition of acetic acid (to destroy the excess of reductant) and dialysis, the recovered material (5 mg) was partially hydrolyzed with 100 mmol/l trifluoroacetic acid (TFA, 1 ml) at 100 °C for 20 mins, and then carbonyl-reduced with $NaBD_4$ (10 mg) in water (1 ml) at 22 °C for 2 hrs. The mixture was divided into two parts. One was acetylated with acetic anhydride-pyridine (1:1) at 100 °C for 30 mins and the other was re-methylated with methyl iodide, hydrolyzed with 2 mol/l TFA (100 °C, 1 hr), and acetylated. Both samples were analyzed by GC-MS.

GC-MS was carried out on a Hewlett-Packard Model 5971 A mass selective detector connected to a Hewlett-Packard Model 5890 A chromatograph equipped with a chemically bonded SE-54 fused silica capillary column (25 m × 0.32 mm; Weeke, Muehlheim, FRG).The column temperature program was 160 °C (3 mins) to 305 °C at 3 °C/min. Electron impact (EI) mass spectra were recorded at 70 eV.

The LPS II of *C. burnetii* in phase II was isolated from purified cells as described previously (Toman *et al.*, 1993). The LPS II was permethylated, carboxyl-reduced and hydrolyzed under mild conditions. The hydrolyzate was carbonyl-reduced and divided into two portions. One portion was acetylated and directly analyzed by GC-MS. Isomeric 1,2,6-tri-O-acetyl-3-deoxy-4,5,7,8-tetra-O-methyl(1,1,2-3D)octitols

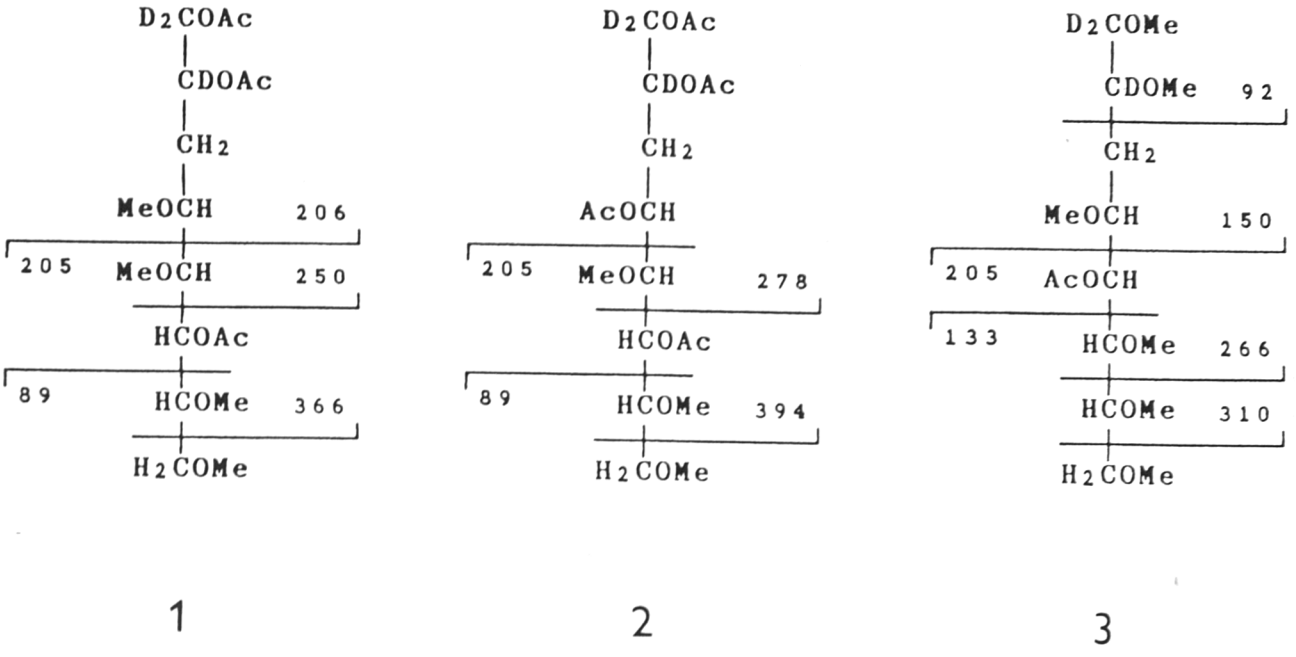

Fig. 1

Fragmentation pattern obtained after EI ionization of 1,2,6-tri-O-acetyl-3-deoxy-4,5,7,8-tetra-O-methyl(1,1,2-3D)octitol (**1**), 1,2,4,6-tetra-O-acetyl-3-deoxy-5,7,8-tri-O-methyl(1,1,2-3D)octitol (**2**) , and 5-O-acetyl-3-deoxy-1,2,4,6,7,8-hexa-O-methyl(1,1,2-3D)octitol (**3**). All octitol derivatives were mixtures of the D-glycero-D-galacto/talo isomers.

Table 1. Characteristic MS fragment ions of the partially methylated and acetylated derivatives of 3-deoxyoctitols

Compound[a]	Base peak (m/z)	Primary fragment ions (m/z) of the moieties								Characteristic and abundant daughter ions (m/z)[b]
		C-1/2	C-1/4	C-1/5	C-1/6	C-1/7	C-5/8	C-6/8	C-7/8	
1	101	–	206	250	–	366	205	–	89	113, 145, 146, 173
2	89	–	–	278	–	394	205	–	89	101,113,116,158,218
3	92	92	150	–	266	310	205	133	–	118, 160, 202, 234

[a] The octitol derivatives were mixtures of the D-glycero-D-galacto/talo isomers.
[b] A maximum of five fragment ions with an intensity exceeding 10% of that of the base peak is given.

(**1**) and 1,2,4,6-tetra-O-acetyl-3-deoxy-5,7,8-tri-O-methyl(1,1,2-3D)octitols (**2**) were identified. Their fragmentation pattern and characteristic fragment ions are given in Fig. 1 and Table 1, respectively. This indicated that the two Kdo residues originally linked by 2–>4 glycosidic bond were released upon mild acid hydrolysis. The other portion was re-methylated, hydrolyzed, and acetylated. Among the products, 5-O-acetyl-3-deoxy-1,2,4,6,7,8-hexa-O-methyl(1,1,2-3D)octitol isomers (**3**) could be identified (Fig. 1, Table 1) after GC-MS. This revealed the presence of the third Kdo unit, proximal to lipid A, which was C-4 substituted by Kdo-(2–>4)-Kdo and C-5 substituted most probably by D-glycero-D-manno-heptose. The latter has been shown to be present in *C. burnetii* LPS (Toman and Kazár, 1991) instead of more common L-glycero-D-mannoheptose found in many enterobacterial LPSs. Thus, the results reported indicate the presence of three Kdo residues in LPS II with the structural arrangement depicted in Fig. 2.

In enterobacterial LPSs, the C-4 position of the lipid A proximal Kdo is substituted by a second Kdo or an alpha-Kdo-(2–>4)-Kdo disaccharide, and the C-5 position by L-glycero-D-mannoheptose, which is also a constitutent of the inner core region (Brade *et al.*, 1988). In the present study, it has been demonstrated for the first time that the

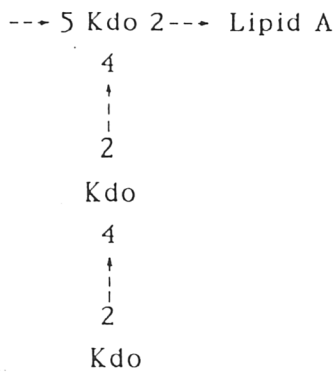

Fig. 2
Structural arrangement of the Kdo residues in the LPS II isolated form Coxiella burnetii in phase II

inner core region of the LPS II of *C. burnetii* contains three Kdo residues with a structural arrangement similar to that of enterobacterial LPSs. Further studies on a more detailed structure of LPS II are in progress.

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