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GLC–MS of reduced, acetylated, and methylated (2 → 4)- and (2 → 8)-linked disaccharides of 3-deoxy-D-*manno*-octulopyranosonic acid (Kdo) *

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ABSTRACT

Synthetic α- and β-(2 → 4)- and α- and β-(2 → 8)-linked disaccharides of 3-deoxy-D-*manno*-octulopyranosonic acid (Kdo), of which the synthesis of the β-(2 → 4)-linked compound is described here, were used to develop a simple GLC–MS method for the determination of their anomeric configuration. The GLC and GLC–MS data for the reduced and acetylated or methylated derivatives of the above compounds indicate the α-linked synthetic disaccharides to be identical to those isolated from bacterial lipopolysaccharides.

INTRODUCTION

A characteristic feature of the core region[1] of lipopolysaccharides (LPS) of Gram-negative bacteria is the presence of at least one residue of 3-deoxy-D-*manno*-octulopyranosonic acid[2] (Kdo). An α-(2 → 4)-linked Kdo-disaccharide, isolated from enterobacterial LPS[3] and characterised by GLC–MS and NMR spectroscopy[4], has also been identified[5] in many other LPS from different Gram-negative bacteria. In LPS of *Chlamydia*, α-(2 → 4)- and α-(2 → 8)-linked Kdo disaccharides, together with the trisaccharide Kdo-(2 → 8)-Kdo-(2 → 4)-Kdo, were identified[6] by GLC–MS. However, the anomeric configurations of the sugars involved in the last two compounds could be identified[7] only by NMR spectroscopy of the isolated pentasaccharide α-Kdo-(2 → 8)-α-Kdo-(2 → 4)-α-Kdo-(2 → 6)-β-GlcN-(1 → 6)-GlcNol, in which the β-GlcN-(1 → 6)-GlcNol moiety represents the deacylated, dephosphorylated, and reduced GlcN backbone of lipid A. Since this

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approach is laborious and requires large quantities of LPS, we have sought an alternative small-scale method for the determination of the anomeric configurations of the sugar moieties in Kdo oligosaccharides. The position of the linkages can be determined by methylation analysis[8,9], and we now report on a GLC–MS method to determine the anomeric configurations of the sugar moieties in α- and β-(2 → 4)- and α- and β-(2 → 8)-linked Kdo-disaccharides, using synthetic standards.

RESULTS AND DISCUSSION

The syntheses of the reducing α- and β-(2 → 8)-linked and α-(2 → 4)-linked Kdo-disaccharide methyl esters have been reported[10,11]. The β-(2 → 4)-linked Kdo-disaccharide **15** was synthesised as follows. The allyl glycoside[12] **13** was acetylated to give **14** (85%). Removal of the allyl group from **14** was accomplished via isomerisation into the propenyl glycoside with bis(methyldiphenylphosphine)-cyclo-octa-1,5-diene-iridium(I) hexafluorophosphate[13,14] followed by treatment with iodine in aqueous oxolane[15] to give **15** (60%).

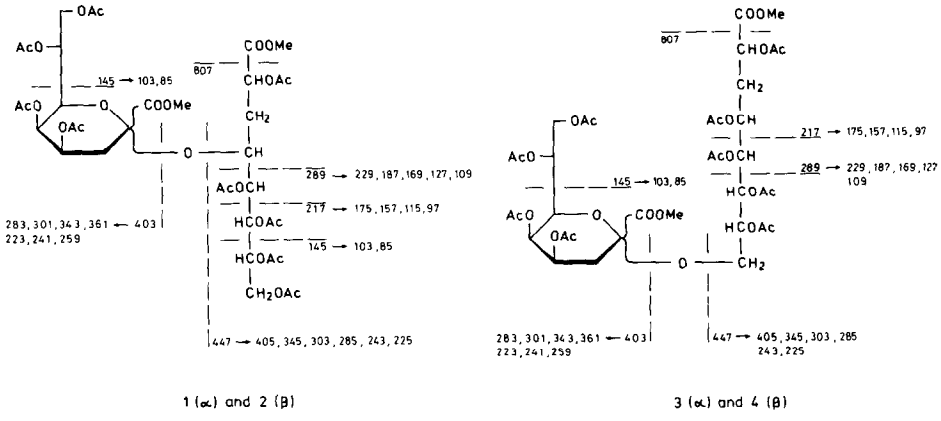
The reduced and acetylated methyl esters **1–4** of the above disaccharides could be separated by GLC. (Table I). The mol wt (866) of each derivative was determined in CI(ammonia)-MS [*m/z* 884, $(M + NH_4)^+$]. As shown in the fragmentation patterns depicted in the formulae, most of the peaks were derived from the ions at *m/z* 403 and 447 (base peak in the spectra of **2–4**) by the loss of either ketene (−42) or acetic acid (−60). Other fragments were obtained by cleavage of the C-1–C-2 (*m/z* 807), C-4–C-5 (*m/z* 289 and the respective secondary ions), and C-5–C-6 bonds (*m/z* 217 and the secondary ions) in the alditol residue, and the C-6′–C-7′ bond of the non-reducing moiety (*m/z* 145 and the secondary ions at *m/z* 103 and 85). Most of the above fragments were present in the spectra of **1–4**; however, there were differences in the intensities which allowed a differentiation between **1** [α-(2 → 4) linkage] and **2** [β-(2 → 4) linkage]. The isomers (D-*glycero*-D-*talo* and D-*glycero*-D-*galacto*, obtained by reduction) of **1** gave different mass spectra, whereas those of the respective isomers of **2–4** were similar. However, the ion at *m/z* 175 was missing from the mass spectrum of the isomer of

TABLE I

Retention times (*T*) [a] of derivatives in GLC

Compound	*T* [a]	Compound	*T* [a]
1	5.93/5.97	**7**	4.20 [b]
2	6.00/6.02	**8**	4.15 [b]
3	6.23/6.26	**9**	3.62 [b]
4	6.27/6.29	**10**	3.49 [b]
5	3.91 [b]	**11**	3.81/3.84
6	3.89 [b]	**12**	3.82/3.84

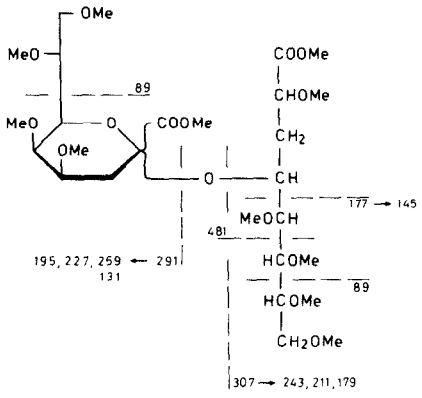
[a] Relative to that of α-D-glucopyranose penta-acetate. [b] The D-*glycero*-D-*talo* and D-*glycero*-D-*galacto* isomers were not separated.



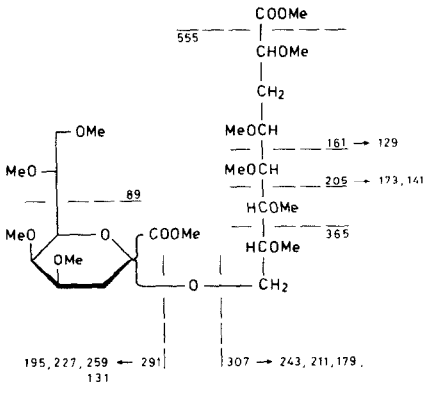
4, which was eluted first, and the ions at m/z 157 and 283 were not present in that eluted second. The mass spectra of **3** and **4** [α- and β-(2 → 8)-linked derivatives] could also be differentiated by the intensities of fragments, e.g., ions at m/z 85, 115, 121, 123, 139, 175, 181, 183, and 229.

The corresponding methylated methyl esters **5–8** could be identified in GLC by their relative retention times (Table I) but the D-*glycero*-D-*talo*/D-*galacto* isomers were not separated. The mol wt (614) of each derivative was determined by CI(ammonia)-MS [m/z 632, $(M + NH_4)^+$]. The fragmentation patterns depicted in the formulae show that most fragments were derived from the ions at m/z 307 and 291 by loss of methanol (−32). Cleavages in the reduced residue of the (2 → 4)-linked compound gave ions at m/z 177 and 145, whereas the (2 → 8)-linked derivative gave ions at m/z 365, 205, 173, 161, 141, and 129. There were some differences in the intensities of the fragment ions in the mass spectra of **5** and **6** [the (2 → 4)-linked compounds], namely, those at m/z 71, 75, 85, 88, 115, 129, 133, 145, 227, 259 and 307. The base peaks had m/z 89 for **5** and m/z 101 for **6**. The ions at m/z 131 and 179 were absent from the latter spectrum. The EI-mass spectra of the (2 → 8)-linked derivatives **7** and **8** were similar; however, the ions at m/z 365 and 555 were absent from the spectrum of **8** and there were differences in intensities of the fragment ions.

The methylated alditols **9** and **10** had different retention times in GLC and could be separated from **11** and **12**. However, the retention times of **11** and **12** were similar, which made it impossible to distinguish between the α and β configuration. The mol wt (586) of **9–12** was determined by CI(ammonia)-MS [m/z 604, $(M + NH_4)^+$] and the fragmentation patterns are depicted in the formulae. The ions at m/z 147 and 191 and their secondary ions (−32) at m/z 115 and 159, respectively, in the mass spectra of the (2 → 8)-linked alditols **11** and **12** were absent from those of the (2 → 4)-linked derivatives **9** and **10**. The mass spectra of **9** and **10** were similar, but there were differences in the intensities of the



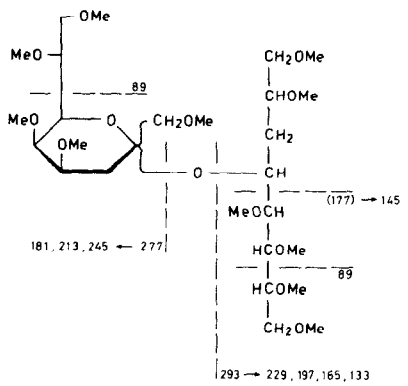
5 (α) and 6 (β)



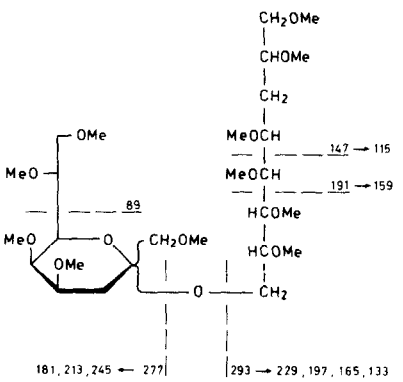
7 (α) and 8 (β)

ions at m/z 59, 71, 75, 89, 133, and 197, and the ion at m/z 229 was absent from the mass spectrum of **9**. The mass spectra of **11** and **12** showed only minor differences and, since the retention times were similar, these derivatives were not useful for the differentiation of the α- and β-(2 → 8)-linked Kdo-disaccharides.

Thus, on the basis of the data reported above, α- and β-(2 → 4)-linked and α- and β-(2 → 8)-linked Kdo-disaccharides can be distinguished by GLC and GLC–MS, preferably of the reduced and methylated methyl esters. Comparison of the data for the reduced and methylated methyl esters of naturally occurring (2 → 4)- (from *Salmonella* LPS) and (2 → 8)-linked (from *Chlamydia* LPS) Kdo-disaccharides with those for the synthetic derivatives **5–8** proved that the linkages were α.



9 (α) and 10 (β)



11 (α) and 12 (β)

EXPERIMENTAL

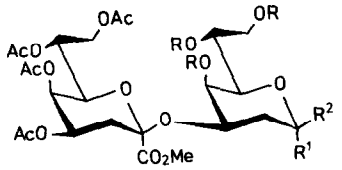
GLC on a capillary column (SE 54) and GLC–MS were performed as described[16]. Temperature programmes in GLC: 160° for 3 min, then 5°/min → 300° for **1–4**; and 150° for 5 min, then 5°/min → 300° for **5–12**.

Methylation was performed according to a modified[8] Hakomori[9] procedure, and the methylated products were purified by the method of Waeghe et al.[17].

O-(*Methyl 4,5,7,8-tetra*-O-*acetyl-3-deoxy-β*-D-manno-*2-octulopyranosylonate)-(2 → 4)-[methyl (allyl 5,7,8-tri*-O-*acetyl-β*-D-manno-*2-octulopyranosid)onate]* (**14**).— To a solution of **13**[14] (18 mg, 0.02 mmol) in pyridine (5 mL) were added acetic anhydride (0.1 mL) and 4-dimethylaminopyridine (1 mg) at 0°. The solution was stirred at room temperature for 15 h, then concentrated. A solution of the residue in CH_2Cl_2 (50 mL) was washed with satd aq $NaHCO_3$, then dried (Na_2SO_4), and the solvent was evaporated. Column (24 × 1 cm) chromatography (toluene–EtOAc, 1:1) of the residue on silica gel afforded **14** (18 mg, 84%), mp 148° (from EtOAc–hexane), $[\alpha]_D^{20}$ + 58° (*c* 1.0, $CHCl_3$). ^{1}H-NMR data ($CDCl_3$): δ 5.87 (m, 1 H, =CH–), 5.35 (bs, 1 H, H-5), 5.26 (bs, 1 H, H-5′), 5.26 (dq, 1 H, $=CH_{2trans}$), 5.17 (dq, 1 H, $=CH_{2cis}$), 5.14 and 5.12 (ddd, 2 H, H-7,7′), 4.91 (ddd, 1 H, $J_{4',5'}$ ~ 2.5, $J_{4',3'eq}$ ~ 4.8, $J_{4',3'ax}$ ~ 13.0 Hz, H-4′), 4.69 (dd, 1 H, $J_{8'a,7'}$) ~ 3.3, $J_{8'a,8'b}$ ~ −12.5 Hz, H-8′a), 4.41 (dd, 1 H, $J_{8a,7}$ ~ 2.5, $J_{8a,8b}$ ~ −12.5 Hz, H-8a), 4.34 (dd, 1 H, $J_{8'b,7'}$ ~ 4.5 Hz, H-8′b), 4.27 (m, 1 H, OCH_2), 4.23 (dd, 1 H, $J_{6',7'}$ ~ 9.5, $J_{6',5'}$ ~ 1.5 Hz, H-6′), 4.11 (dd, 1 H, $J_{6,7}$ ~ 9.5, $J_{6,5}$ ~ 1.5 Hz, H-6), 4.07 (dd, 1 H, $J_{8b,7}$ ~ 2.0 Hz, H-8b), 3.96 (m, 1 H, OCH_2), 3.94 (ddd, 1 H, $J_{4,5}$ ~ 3.0, $J_{4,3eq}$ ~ 5.0, $J_{4,3ax}$ ~ 12.5 Hz, H-4), 3.83 and 3.81 (2 s, each 3 H, 2 CO_2Me), 2.28 (dd, 2 H, H-3*eq*,3′*eq*), 2.06 (t, 1 H, $J_{3ax,3eq}$ ~ 12.0 Hz, H-3*ax*), 2.00 (t, 1 H, $J_{3'ax,3'eq}$ ~ 12.5 Hz, H-3′*ax*), 2.13 (s, 6 H), 2.10 (s, 3 H), 2.08 (s, 3 H), 2.06 (s, 3 H), 2.04 (s, 3 H), 2.00 (s, 3 H), and 1.98 (s, 3 H) (7 Ac).

Anal. Calcd for $C_{35}H_{48}O_{22}$: C, 51.22; H, 5.89. Found: C, 51.07; H 5.70.

O-(*Methyl 4,5,7,8-tetra*-O-*acetyl-3-deoxy-β*-D-manno-*2-octulopyranosylonate)-(2 → 4)-(methyl 5,7,8-tri*-O-*acetyl-3-deoxy-α*-D-manno-*2-octulopyranosonate)* (**15**).— A



13 R=H , R^1=CO_2Me, R^2=OAll
14 R=Ac, R^1=CO_2Me, R^2=OAll
15 R=Ac, R^1=OH , R^2=CO_2Me

solution of $[Ir(COD)(PMePh_2)_2]PF_6$ (5 mg) in dry oxolane (5 mL) was degassed, placed under O_2-free N_2, and degassed once more. The catalyst was activated in an H_2 atmosphere for 5 min. The solution was degassed, added to a solution of **14** (14 mg) in dry oxolane (3 mL), stirred for 4 h at room temperature, and then concentrated. A solution of the residue in CH_2Cl_2 (20 mL) was washed with satd aq $NaHCO_3$, then dried (Na_2SO_4), and the solvent was evaporated. A solution of the residue in oxolane–water (4:1, 5 mL) was treated with I_2 (10 mg) for 10 min at room temperature, then diluted with CH_2Cl_2 (50 mL), washed with aq 20% $NaHSO_3$ and satd aq $NaHCO_3$, dried (Na_2SO_4), and concentrated. Column chromatography (toluene–EtOAc, 1:1) of the residue gave **15**, isolated as a syrup (8 mg, 60%), $[\alpha]_D^{20}$ + 67° (*c* 0.8, $CHCl_3$); ^{1}H-NMR data ($CDCl_3$): δ 5.44 (bs, 1 H, H-5), 5.28 (bs, 1 H, H-5′), 5.20 (ddd, 1 H, $J_{7',6'}$ ~ 10.0 Hz, H-7′), 5.06 (ddd, 1 H, $J_{7,6}$ ~ 10.0 Hz, H-7), 4.94 (ddd, 1 H, $J_{4',5'}$ ~ 3.0, $J_{4',3'eq}$ ~ 5.0, $J_{4',3'ax}$ ~ 12.5 Hz, H-4′), 4.53 (dd, 1 H, $J_{8'a,7'}$ ~ 4.0, $J_{8'a,8'b}$ ~ 12.0 Hz, H-8′a), 4.40 (ddd, 1 H, $J_{4,3ax}$ ~ 12.0, $J_{4,3eq}$ ~ 4.5, $J_{4,5}$ ~ 3.7 Hz, H-4), 4.40 (dd, 1 H, $J_{8a,7}$ ~ 2.5, $J_{8a,8b}$ ~ 12.0 Hz, H-8a), 4.30 and 4.25 (dd, 2 H, $J_{6,5}$ ~ 1.0, $J_{6,7}$ ~ 9.5 Hz, H-6,6′), 4.29 (dd, 1 H, $J_{8'b,7'}$ ~ 2.0 Hz, H-8′b), 4.16 (dd, 1 H, $U_{8b,7}$ ~ 4.5 Hz, H-8b), 3.97 (d, 1 H, J ~ 2.5 Hz, OH), 3.86 and 3.80 (2 s, each 3 H, 2 CO_2Me), 2.39 (t, 1 H, $J_{3ax,3eq}$ ~ 12.5 Hz, H-3*ax*), 2.28 (dd, 1 H, $J_{3'eq,3'ax}$ ~ 12.5 Hz, H-3′*eq*), 2.13 (s, 6 H), 2.09 (s, 3 H), 2.07 (s, 6 H), 2.03 (s, 3 H), and 1.99 (s, 3 H) (7 Ac), 2.03 (t, 1 H, H-3′*ax*), 1.76 (dd, 1 H, H-3*eq*).

Anal. Calcd for $C_{32}H_{44}O_{22}$: C, 49.23; H, 5.68. Found: C, 48.54; H, 5.83.

Selective carbonyl-reduction of the α- and β-(2 → 4)- and α- and β-(2 → 8)-linked Kdo disaccharide methyl esters with $NH_3 \cdot BH_3$ in methanol, followed by concentration to dryness and then *O*-acetylation in 1:1 pyridine–acetic anhydride, catalysed by 4-dimethylaminopyridine (16 h, room temperature), afforded **1–4**. *O*-Deacetylation (methanolic 0.25 M sodium methoxide, 15 min, room temperature) of **1–4** followed by methylation yielded **5–8**. Carboxyl-reduction of **5–8** with $LiAlH_4$ in ether followed by methylation gave **9–12**. The *T* values in GLC of **1–12** are listed in Table I. The preparations of **1–12** were performed on small amounts of material sufficient for GLC and GLC–MS.

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