Carbohydrate Research, 179 (1988) 289–299
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

ISOLATION AND CHEMICAL ANALYSIS OF 7-*O*-(2-AMINO-2-DEOXY-α-D-GLUCOPYRANOSYL)-L-*glycero*-D-*manno*-HEPTOSE AS A CONSTITUENT OF THE LIPOPOLYSACCHARIDES OF THE UDP-GALACTOSE EPIMERASE-LESS MUTANT J-5 OF *Escherichia coli* AND *Vibrio cholerae**

WIESLAV KACA†, JAQUELINE DE JONGH-LEUVENINK‡, ULRICH ZÄHRINGER, ERNST TH. RIETSCHEL, HELMUT BRADE**,

Forschungsinstitut Borstel, Institut für Experimentelle Biologie und Medizin, Parkallee 22, D-2061 Borstel (F.R.G.)

JAN VERHOEF,

Laboratory of Microbiology, State University of Utrecht, Catharijnesingel 59, NL-3511 GG Utrecht (The Netherlands)

AND VOLKER SINNWELL

Institut für Organische Chemie der Universität, Martin Luther King Platz 6, D-2000 Hamburg (F.R.G.)

(Received October 21st, 1987; accepted for publication, December 14th, 1987)

ABSTRACT

Methyl 7-O-(2-amino-2-deoxy-α-D-glucopyranosyl)-L-*glycero*-D-*manno*-hepto pyranoside (**1**) was released from the lipopolysaccharide of the UDP-galactose epimerase-less mutant J-5 of *Escherichia coli* by methanolysis and isolated by high-voltage paper electrophoresis. Its chemical structure was determined by chemical analysis, deamination with nitrous acid, g.l.c.–m.s., and ^{1}H- and ^{13}C-n.m.r. spectroscopy performed on its acetylated derivative. The disaccharide moiety of **1** was also detected in lipopolysaccharides of *Vibrio cholerae*.

INTRODUCTION

Lipopolysaccharides (LPS) are characteristic constituents of the Gram-negative cell wall where they are located in the outer leaflet of the outer membrane. They represent the endotoxins of Gram-negative bacteria[1] of which they are the major surface antigens and useful serological markers. Further, they have been investigated for their potential use in the induction of protective antibodies against endotoxicity or infection. Many investigators have reported on cross-reactive and cross-protective poly- and mono-clonal antibodies against certain rough-mutant bacteria, *i.e.*,

*Dedicated to Professor Bengt Lindberg.

†Present address: Institute of Microbiology, University of Lodz, ul. Banacha 12/16, PL-90-237 Lodz, Poland.

‡Present address: Laboratory of Microbiology, State University of Utrecht, Catharijnesingel 59, NL-3511 GG Utrecht, The Netherlands.

**Author for correspondence.

0008-6215/88/$03.50

the Re mutant of *Salmonella minnesota* R595 (Re chemotype)[2] and the UDP-galactose epimerase-less mutant J-5 of *Escherichia coli* (Rc chemotype)[3]. These antibodies were assumed to be directed against common structures in the inner core region of the LPS molecule which, in *Enterobactereaceae, Vibrionaceae, Pseudomonadaceae,* and many others, consists of heptose (mainly L-*glycero*-D-*manno* and D-*glycero*-D-*manno*) and 3-deoxy-D-*manno*-octulosonic acid (KDO). This region has been well characterised chemically in enterobacterial rough mutants[4-6]. However, little is known about the chemistry of the J-5 LPS. It has been reported[7] that this LPS exhibits a considerable heterogeneity in its saccharide moity. Among the partial structures described was a disaccharide composed of 2-amino-2-deoxyglucose and heptose which was later detected also in the LPS of *Bordetella pertussis*[8], *Shigella flexneri*[9], *Aeromonas hydrophila*[10,11], and *Vibrio ordalii*[12]. This structural element is seemingly widely distributed in bacterial LPS, and we now report the identification of the disaccharide isolated from the LPS of J-5 and show that it occurs also in the LPS of three serotypes of *Vibrio cholerae.*

EXPERIMENTAL

Bacteria and bacterial lipopolysaccharide (LPS). — Two specimens of the UDP-galactose epimerase-less mutant J-5 of *E. coli* were used, which were derived from the original strain[3]. One was obtained through the courtesy of M. P. Glauser (Centre Hôpitalier Universitaire Vaudois Lausanne, Switzerland) and has been maintained in Utrecht; the other was obtained from H. D. Hungerer (Behring Werke Marburg, F.R.G.). Bacteria were grown in a fermenter (80 L), killed with phenol (0.5%), and centrifuged. The sedimented bacteria were washed successively with ethanol, acetone (twice), and ether, and then dried. LPS was isolated (3.5%) from dry bacteria by the phenol–chloroform–light petroleum method[13]. The crude LPS was purified by electrodialysis[14] followed by a second extraction with phenol–chloroform–light petroleum.

The following strains of *V. cholerae* were cultivated: 95R (rough mutant of serotype Ogawa 162), 569B (serotype Inaba), and H11 (non-agglutinating strain). LPS was obtained from these strains, as described above, in yields of 3–4% of the dry weight.

Analytical methods. — Neutral sugars were determined as their alditol acetates by g.l.c. after hydrolysis (100° for 48 h) in 0.1M HCl, using D-xylose as the internal standard. Fatty acids were identified as their methyl esters by g.l.c. and g.l.c.–m.s. after acid hydrolysis (4M HCl, 100° for 4 h) followed by methanolysis (2M HCl, 85° for 16 h). For quantitative analysis, heptadecanoic and 3-hydroxydodecanoic acid were added as internal standards, and 3-hydroxy fatty acids were derivatised by reaction with *tert*-butylchlorodimethylsilane. 2-Amino-2-deoxy-D-glucose was identified using an amino acid analyser (no other amino sugars were detected) and quantified by the modified Morgan–Elson reaction[15] after hydrolysis with HCl (4M, 100° for 16 h). KDO was determined by the thiobarbiturate assay[16].

Phosphorus was determined according to Lowry[17].

General methods. — Reduction with sodium borohydride or sodium borodeuteride was performed conventionally, as was acetylation with pyridine–acetic anhydride (1:1). For *N*-acetylation, solutions of samples (100 μg) in saturated aqueous sodium hydrogencarbonate (50 μL) were stirred with acetic anhydride (2 μL) for 30 min at room temperature. High-voltage paper electrophoresis was carried out at 40 V/cm in pyridine–acetic acid–formic acid–water (1:10:1.5:90; pH 2.8) with detection by alkaline silver nitrate for reducing sugars, ninhydrin (0.2% in acetone) for free amino groups, a molybdate reagent for phosphorus, and the thiobarbiturate reagent for KDO.

Liberation of the disaccharide glycoside **1** *from LPS by methanolysis.* — Aliquots of LPS (2 mg each) in methanolic 2M HCl (1 mL) were kept for various lengths of time at 85°. The samples were concentrated several times under reduced pressure with methanol, and the residues were then dried over sodium hydroxide. Maltose was added to the dry samples as internal standard, followed by acetylation and g.l.c.-m.s.

Liberation of the disaccharide **2** *from LPS by hydrolysis.* — Aliquots of LPS (2mg each) were hydrolysed (120°) in 2M trifluoroacetic acid (500 μL) for various lengths of time. The samples were concentrated and toluene was distilled from the residue several times under reduced pressure. Maltose was added as internal standard to the dry samples, followed by reduction, acetylation, and g.l.c.-m.s.

Derivatisation of **1** *and* **2** *for g.l.c.-m.s.* — Methylation or acetylation of **1** yielded methyl 7-*O*-(2-deoxy-2-dimethylamino-3,4,6-tri-*O*-methyl-α-D-glucopyranosyl)-2,3,4,6-tetra-*O*-methyl-L-*glycero*-α-D-*manno*-heptopyranoside (**3**) and methyl 7-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-2,3,4,6-tetra-*O*-acetyl-L-*glycero*-α-D-*manno*-heptopyranoside (**4**), respectively. *N*-Acetylation of **1** followed by methylation afforded methyl 7-*O*-(2-deoxy-3,4,6-tri-*O*-methyl-2-*N*-methylacetamido-α-D-glucopyranosyl)-2,3,4,6-tetra-*O*-methyl-L-*glycero*-α-D-*manno*-heptopyranoside **5**. Hydrolysis of **3** followed by reduction and acetylation gave 1,5-di-*O*-acetyl-7-*O*-(2-deoxy-2-dimethylamino-3,4,6-tri-*O*-methyl-α-D-glucopyranosyl)-2,3,4,6-tetra-*O*-methyl-L-*glycero*-D-*manno*-heptitol (**6**), whereas the same reaction sequence performed on **5** gave 1,5-di-*O*-acetyl-7-*O*-(2-deoxy-3,4,6-tri-*O*-methyl-2-*N*-methylacetamido-α-D-glucopyranosyl)-2,3,4,6-tetra-*O*-methyl-L-*glycero*-D-*manno*-heptitol (**7**). Reduction of **2** followed by methylation yielded 7-*O*-(2-deoxy-2-dimethylamino-3,4,6-tri-*O*-methyl-α-D-glucopyranosyl)-1,2,3,4,5,6-hexa-*O*-methyl-L-*glycero*-D-*manno*-heptitol (**8**). 7-*O*-(2-Deoxy-3,4,6-tri-*O*-methyl-2-*N*-methylacetamido-α-D-glucopyranosyl)-1,2,3,4,5,6-hexa-*O*-methyl-L-*glycero*-D-*manno*-heptitol (**9**) was obtained from **2** by *N*-acetylation, reduction, and methylation. 7-*O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-1,2,3,4,5,6-hexa-*O*-acetyl-L-*glycero*-D-*manno*-heptitol (**10**) was prepared from **2** by reduction and acetylation.

Deamination with nitrous acid. — To separate solutions of LPS (1 mg) and **1** (100 μg) in water (100 μL) were added freshly prepared aqueous 5% sodium nitrite

(100 μL) and aqueous 33% acetic acid (100 μL). Each mixture was stirred for 1 h at room temperature, then passed through Amberlite IRA-120 (H^+) resin, and concentrated to dryness. The residue was reduced with sodium borohydride, acetylated, and analysed by g.l.c.-m.s. 2-Amino-2-deoxy-D-glucose was used as a control.

G.l.c. and g.l.c.-m.s. — G.l.c. was performed with a Varian 3700 gas chromatograph equipped with a flame-ionisation detector and a fused-silica capillary column (25 m × 0.32 mm i.d.) with chemically bonded SE-54 (0.2 μm) (Weeke, Mühlheim); the carrier gas was H_2 (1.0 bar). Temperature programme: 150° for 5 min, 5°/min → 300°. G.l.c.-m.s. was carried out on a Hewlett-Packard instrument (Model 5985) equipped with an SE-54 column and an HP-1000 data system. E.i.-mass spectra were recorded at 70 eV and c.i.-mass spectra were obtained with ammonia as reactant gas. The ion-source temperature was 200°. Methylation analysis was performed on **5** by hydrolysis in M trifluoroacetic acid (16 h at 100°) followed by reduction and acetylation.

Isolation and purification of **1**. — LPS of *E. coli* J-5 (800 mg) was methanolysed (2M HCl, 60 mL; 85° for 16 h) with constant stirring. The volume was then reduced to ~30 mL, and the solution was diluted with water (30 mL), neutralised with sodium hydroxide, and concentrated to dryness. A solution of the residue in water (30 mL) was extracted with dichloromethane (3 × 30 mL). The aqueous phase was lyophilised, and a solution of the residue in water (2 mL) was subjected to preparative high-voltage paper electrophoresis (40 V/cm) in pyridine–acetic acid–formic acid–water (1:10:1.5:90). The fraction with M_{GlcN} 0.63 was eluted from the paper and subjected again to high-voltage paper electrophoresis. Lyophilisation then yielded 14.8 mg of amorphous **1**, $[\alpha]_D$ + 37° (*c* 0.48, water). The product **1** (7.8 mg) was treated conventionally at room temperature with pyridine–acetic anhydride (2:1) and a catalytic amount of 4-dimethylaminopyridine. The reaction was monitored by t.l.c. on Silica Gel 60 F_{254} (Merck) with *A*, chloroform–methanol (9:1); and *B*, toluene–ethanol (10:1); and detection by charring with sulfuric acid.

TABLE I

CHEMICAL COMPOSITION OF *E. coli* J5 LPS

Sample[a]	*Constituent*[b] *(nmol/mg)*							
	GlcN	*KDO*[c]	*P*	*Glc*	*Hep*	*C12:0*	*C14:0*	*C14:0(3-OH)*
1	564 (109)[d]	459 (102)	1250 (39)	282 (51)	502 (105)	198 (40)	153 (35)	897 (219)
2	585 (105)	465 (111)	1237 (38)	343 (62)	472 (99)	177 (35)	189 (43)	867 (212)

[a]LPS was prepared from two independently grown specimens of the same bacterial strain of *E. coli* J5. [b]P, phosphate; Hep, L-*glycero*-D-*manno*-heptose, C12:0, dodecanoic acid; C14:0, tetradecanoic acid; C14:0(3-OH), 3-hydroxytetradecanoic acid. [c]3-Deoxy-D-*manno*-octulosonic acid determined after hydrolysis in M HCl (1 h at 100°). [d]Values are expressed in μg/mg.

Acetylation was completed after 6 h. The acetylated disaccharide **4** had R_F 0.9 (solvent *A*), and 0.29 and 0.24 (solvent *B*) corresponding to the methyl α- and β-glycoside in the ratio of ~1:7 (data not shown). The α,β-mixture was fractionated by semi-preparative h.p.l.c. on a column (300 × 7 mm i.d.) of Nucleosil (5 μm) under isocratic conditions[18] to give **4** (*T* 12.5 min; *cf.* 15.0 for the β anomer).

^{1}H- and ^{13}C-n.m.r. spectroscopy. — Using a solution of **4** in C_6D_6, ^{1}H- (400 MHz) and ^{13}C-spectra (100.62 MHz) ($CDCl_3$, 77 p.p.m.) were recorded[18] on a Bruker WM-400 instrument.

RESULTS AND DISCUSSION

Chemical composition of J-5 LPS. — Chemical analysis of the LPS derived from two independently cultivated strains of *E. coli* J-5 yielded the data shown in

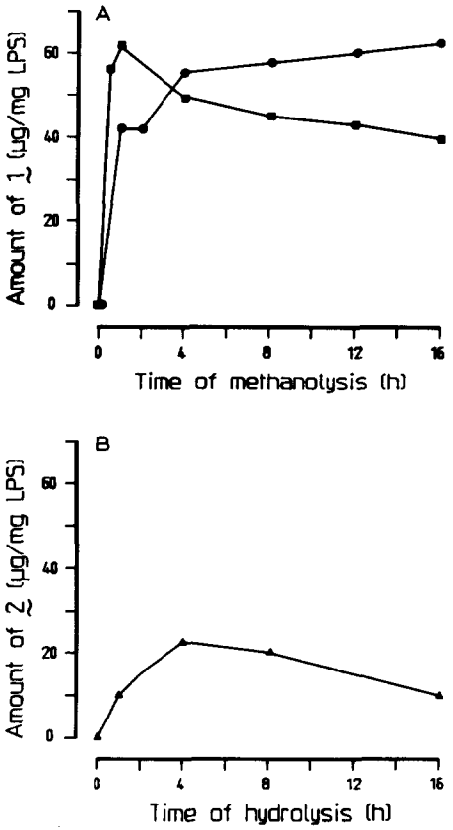
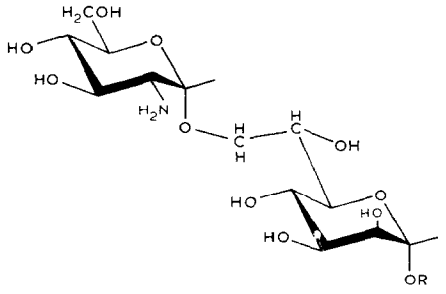
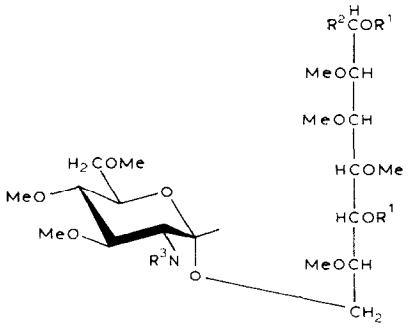


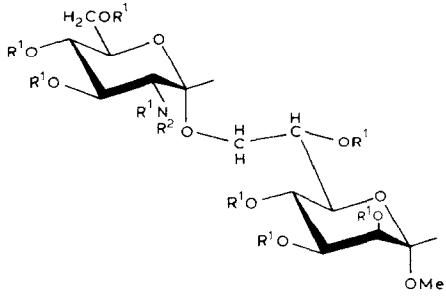
Fig. 1. Kinetics of the release of *A*, the disaccharide glycoside **1** from the LPS of *Escherichia coli* J-5 by methanolysis in 2M (●) and 4M (■) HCl at 85°; and of *B*, the disaccharide **2** by hydrolysis of LPS in 2M trifluoroacetic acid at 120° (Δ).



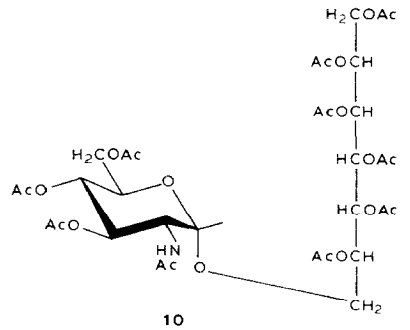
1 R = Me
2 R = H



6a R^1 = Ac, R^2 = H, R^3 = Me
6b R^1 = Ac, R^2 = D, R^3 = Me
7a R^1 = Ac, R^2 = H, R^3 = Ac
7b R^1 = Ac, R^2 = D, R^3 = Ac
8 R^1 = Me, R^2 = H, R^3 = Me
9 R^1 = Me, R^2 = H, R^3 = Ac



3 R^1 = Me, R^2 = Me
4 R^1 = Ac, R^2 = H
5 R^1 = Me, R^2 = Ac



10

Table I. Glucose and L-*glycero*-D-*manno*-heptose were detected as the sole neutral sugars, confirming the Rc chemotype of this LPS. The constituents analysed accounted for 71% of the LPS. The contamination of each sample with protein was < 1% (determined by amino acid analysis) and nucleic acids were absent (determined by the absence of ribose). The amounts of 2-amino-2-deoxy-D-glucose and heptose are minimum values since, under the conditions of hydrolysis employed for their determination, **1** was still detected.

Release, isolation, and purification of the disaccharide glycoside **1** *from J-5 LPS.* — The kinetics of hydrolysis were studied using 2M trifluoroacetic acid at 120° and methanolic 2M and 4M HCl at 85° followed by appropriate derivatisation for g.l.c. The results are shown in Fig. 1. Whereas a total of 22 μg/mg of LPS were detected after hydrolysis with trifluoroacetic acid (Fig. 1B), 63 μg/mg of LPS were

TABLE II

G.L.C. AND G.L.C.–M.S. DATA FOR **3–10**.

Compound	*Mol. wt.*[a]	*Retention time*[b]	*Characteristic e.i.-fragments*[c] *m/z (% of base peak)*
3	511	2.42	101(100), 114(43.3), 115(66.7), 232(8.9), 248(5.6), 263(1.1)
4	721	3.60	101(100), 115(44.4), 143(70), 330(17.8), 275(75.6)
5	539	3.00	75(44.4), 87(46.7), 88(91.1), 101(71.1), 115(15.6), 129(100), 142(73.3), 199(34.4), 228(13.3), 231(13.3), 263(44.4), 333(14.4)
6b	584	2.78	86(21.6), 101(100), 102(25.8), 114(58.8), 115(45.4), 118(15.5), 162(4.1), 200(3.1), 232(10.3)
7b	612	3.28	87(40), 88(40), 101(40), 102(53.3), 118(60), 129(100), 142(76.6), 162(13.2), 228(26.4), 260(15.6), 322(1.1), 336(44.4)
8	527	2.38	101(100), 114(88.9), 115(44.4), 232(8.9), 248(3.3), 279(1.1)
9	555	2.96	71(53.3), 87(42.2), 88(44.4), 89(42.2), 101(100), 115(45.6), 129(60), 142(48.9), 228(37.8), 260(17.8), 279(40)
10	793	3.78	84(100), 101(91.3), 115(43.5), 129(52.2), 143(58.7), 330(17.4), 433(13), 447(48.9)

[a]Determined by c.i. (ammonia)-m.s. on the basis of peaks at m/z for $(M+1)^+$ and $(M+18)^+$. [b]Relative to that of 1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranose, using a fused-silica capillary column (25 m × 0.32 mm i.d.) with chemically bonded SE-54, a temperature programme of 150° for 5 min and then 5°/min → 300°, and H_2 as carrier gas (1.0 bar). [c]Determined by e.i.-m.s. at 70 eV.

found after methanolysis (Fig. 1A). Since methanolysis in 4M HCl gave a peak maximum and that in 2M HCl yielded a plateau value, the latter conditions were chosen for a preparative scale experiment. Thus, from 800 mg of LPS, 14.8 mg of **1** were isolated and purified by high-voltage paper electrophoresis. The compound had M_{GlcN} 0.63 which accords with that reported[10]. Hydrolysis of **1** with 2M trifluoroacetic acid gave the disaccharide **2**.

G.l.c. and g.l.c.–m.s. of **3–10** *prepared from* **1** *and* **2**. — The retention in g.l.c. (relative to that of D-glucose penta-acetate), the molecular weights [determined by c.i.(ammonia)-m.s.], and the characteristic fragment ions (obtained after e.i.-m.s.) are summarised in Table II. These data confirmed that **2** was a 2-amino-2-deoxy-D-glucosyl-heptose. The e.i.-spectra of **7** and **10** suggested a 7-linkage since fragment

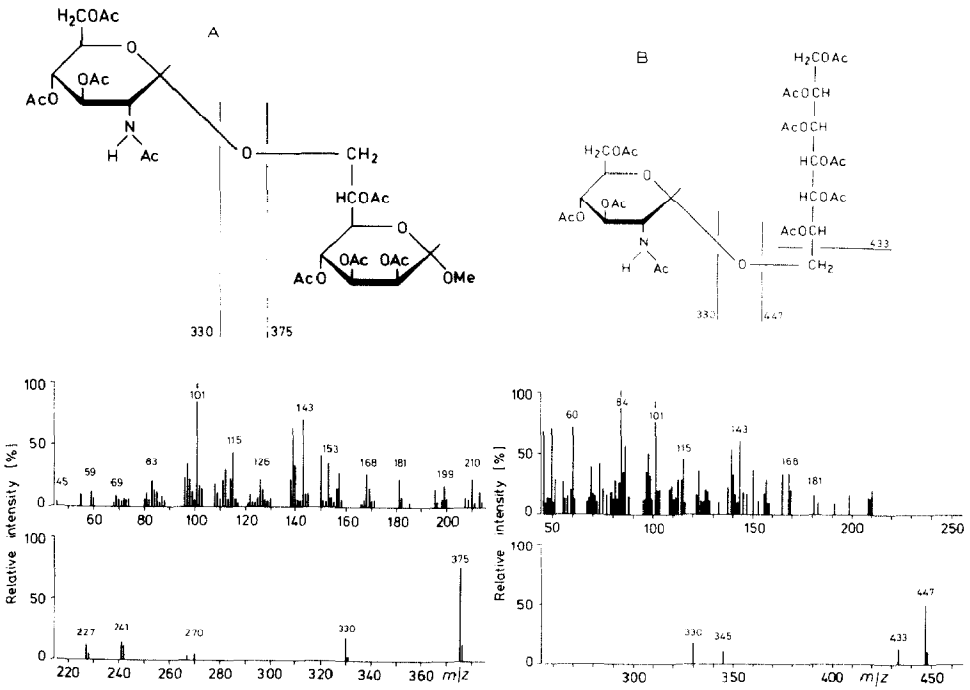


Fig. 2. E.i. spectra (70 eV) of *A*, methyl 7-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-2,3,4,6-tetra-*O*-acetyl-L-*glycero*-α-D-*manno*-heptoside; and *B*, 7-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl)-1,2,3,4,5,6-hexa-*O*-acetyl-L-*glycero*-D-*manno*-heptitol.

ions corresponding to the C-1/6 moiety were observed at *m/z* 321 for **7a** (*m/z* 322 for the deuterio derivative **7b**) and at *m/z* 433 for **10**. Further evidence for a 7-linkage was obtained by methylation analysis of **5**, which yielded 1,5-di-*O*-acetyl-2-deoxy-3,4,6-tri-*O*-methyl-2-*N*-methylacetamidoglucitol and 1,5,7-tri-*O*-acetyl-2,3,4,6-tetra-*O*-methylheptitol. The heptitol hepta-acetate obtained from **1** after hydrolysis, reduction, and acetylation co-migrated in g.l.c with authentic L-*glycero*-D-*manno*-heptitol hepta-acetate and was separated from the D-*glycero*-D-*manno* isomer.

The spectra of **6b** and **7b** were identical to those published[11]. The derivatives containing a dimethylamino group exhibited poor fragmentation, and the main fragments were those from cleavage of the glycosidic bond. Nevertheless, the detection of these derivatives by g.l.c.-m.s. provides a method suitable for searching for disaccharide **2**. Compounds **4** (obtained by methanolysis of LPS followed by acetylation) and **10** (obtained by hydrolysis with trifluoroacetic acid, then reduction, and acetylation) are specially suited for this purpose. The spectra of these compounds are shown in Fig. 2.

Deamination with nitrous acid. — When LPS was deaminated and the products were reduced and acetylated, only small amounts (~0.5 μg/mg of LPS) of

anhydromannitol tetra-acetate were detected, indicating that the amino group, at least partially, was unsubstituted in LPS. No glucosyl-anhydromannitol derivative was detected, which could be expected from the partial substitution of 2-amino-2-deoxy-D-glucose with glucose in position 4 (ref. 7). However, deamination of **1** followed by reduction and acetylation gave anhydromannitol tetra-acetate and methyl penta-*O*-acetylheptoside in equimolar amounts.

^{1}H- and ^{13}C-n.m.r. spectroscopy. — In the ^{1}H-n.m.r. spectrum of **4**, the $J_{1',2'}$ and $J_{1,2}$ values of 3.8 and 1.5 Hz, respectively, were consistent with an α linkage and a methyl α-glycoside (Table III). These findings were further supported by the ^{13}C-n.m.r data (Table IV) and the proton-coupled ^{13}C-n.m.r. data which showed $^1J_{C\text{-}1,H\text{-}1}$ and $^1J_{C\text{-}1',\ H\text{-}1'}$ values of 176.7 and 173.5 Hz, respectively, indicating[19,20] both sugar residues to be α. In addition, the $J_{2,3}$ and $J_{3,4}$ values of 3.4 and 10 Hz, respectively, indicated the *manno* configuration of the heptopyranose. 7-Substitution of the heptopyranose was indicated by a shift of the resonances of H-7a (3.80 p.p.m.) and H-7b (3.61 p.p.m.) to higher fields compared to those in 1,2,3,4,6,7-hexa-*O*-acetyl-L-*glycero*-D-*manno*-heptopyranose[21] (H-7a, 4.28; H-7b, 4.18 p.p.m.).

The isolation of disaccharide **2** *from the LPS of* V. cholerae. — When the LPS of three serotypes of the human pathogen *V. cholerae* were investigated as described above, **3–10** were identified by g.l.c. and g.l.c.–m.s.

TABLE III

400-MHz ^{1}H-N.M.R. DATA[a] FOR METHYL 7-*O*-(2-ACETAMIDO-3,4,6-TRI-*O*-ACETYL-2-DEOXY-α-D-GLUCOPYRANOSYL)-2,3,4,6-TETRA-*O*-ACETYL-L-*glycero*-α-D-*manno*-HEPTOPYRANOSIDE (**4**)

Assignment	δ	J*(Hz)*
GlcNAc		
NH-2′	5.87(d)	$J_{2',NH}$ 9.3
H-3′	5.50(dd)	$J_{3',4'}$ 9.6
H-4′	5.33(dd)	$J_{4',5'}$ 10.2
H-1′	4.73(d)	$J_{1',2'}$ 3.8
H-2′	4.57(ddd)	$J_{2',3'}$ 10.4
H-6′a	4.28(dd)	$J_{6a',5'}$ 5.0
H-6′b	4.16(dd)	$J_{6'b,6'a}$ 12.0
H-5′	3.47(dd)	$J_{5',6'b}$ 2.5
Hep		
H-4	5.71(dd)	$J_{4,5}$ 10.0
H-3	5.63(dd)	$J_{3,4}$ 10.0
H-2	5.53(dd)	$J_{2,3}$ 3.4
H-6	5.41(ddd)	$J_{6,7a}$ 8.0
H-1	4.47(d)	$J_{1,2}$ 1.5
H-5	3.91(dd)	$J_{5,6}$ 2.5
H-7a	3.80(dd)	$J_{7a,7b}$ 12.0
H-7b	3.61(dd)	$J_{6,7b}$ 3.5

[a]Other signals: 1.85, 1.80, 1,80, 1,74, 1,71, 1,70, 1,67, 1,65 (8 s, 8 OAc), and 2.95 p.p.m. (s, OMe).

TABLE IV

^{13}C-N.M.R. CHEMICAL SHIFTS OF METHYL 7-*O*-(2-ACETAMIDO-3,4,6-TRI-*O*-ACETYL-2-DEOXY-α-D-GLUCOPYRANOSYL)-2,3,4,6-TETRA-*O*-ACETYL-L-*glycero*-α-D-*manno*-HEPTOPYRANOSIDE (**4**)[a]

Assignment[b]	*Signal (p.p.m.)*	*Assignment*	*Signal (p.p.m.)*
C-1	99.3	C-4′	68.9
C-1′	98.6	C-5′	68.7
C-3′	71.7	C-7	67.0
C-2	70.0	C-4	65.5
C-3	69.6	C-6′	62.4
C-5	69.3	OCH	55.2
C-6	69.0	C-2′	52.3

[a]Recorded at 100.62 MHz with C_6D_6 as internal standard (128 p.p.m.). [b]Assignment by ^{1}H/^{13}C-n.m.r. heteronuclear COSY spectroscopy.

The results of the present investigation provide a basis for understanding the immunoreactive properties of bacterial LPS at the molecular level. The disaccharide glycoside **1** is being evaluated as an antigenic determinant.

ACKNOWLEDGMENTS

We thank Veronika Susott for technical assistance and H. Moll for recording the mass spectra. The financial support of the Deutsche Forschungsgemeinschaft (HB, Br731/4), the Fonds der Chemischen Industrie (EThR), and the Dutch Prevention Fond (JV no 28-964) is gratefully acknowledged.

REFERENCES

1 R. A. PROCTOR, IN E. T. RIETSCHEL (Ed.), *Handbook of Endotoxin: Vol. I, Chemistry of Endotoxin*, Elsevier, Amsterdam, 1984.
2 O. LÜDERITZ, A. M. STAUB, AND O. WESTPHAL, *Bacteriol. Rev.*, 30 (1966) 192–255.
3 A. D. ELBEIN AND C. HEATH, *J. Biol. Chem.*, 240 (1965) 1919–1925.
4 H. BRADE, H. MOLL, AND E. T. RIETSCHEL, *Biomed. Mass Spectrom.*, 12 (1985) 602–609.
5 H. BRADE AND E. T. RIETSCHEL, *Eur. J. Biochem.*, 145 (1984) 231–236.
6 A. TACKEN, E. T. RIETSCHEL, AND H. BRADE, *Carbohydr. Res.*, 149 (1986) 279-291.
7 N. A. FULLER, M. -C. WU, R. G. WILKINSON, AND E. C. HEATH, *J. Biol. Chem.*, 248 (1973) 7938–7950.
8 R. CHABY AND L. SZABO, *Eur. J. Biochem.*, 70 (1976) 115–122.
9 E. KATZENELLENBOGEN AND E. ROMANOWSKA, *Eur. J. Biochem.*, 113 (1980) 205–211.
10 J. H. BANOUB, Y.-M. CHOY, F. MICHON, AND D. H. SHAW, *Carbohydr. Res.*, 114 (1983) 267–276.
11 J. B. BANOUB, F. MICHON, D. H. SHAW, AND R. ROY, *Carbohydr. Res.*, 128 (1984) 203–216.
12 J. H. BANOUB AND H. J. HODDER, *Can. J. Biochem. Cell Biol.*, 63 (1985) 1199–1205.
13 C. GALANOS, O. LÜDERITZ, AND O. WESTPHAL, *Eur. J. Biochem.*, 9 (1969) 245–249.
14 C. GALANOS AND O. LÜDERITZ, *Eur. J. Biochem.*, 54 (1975) 603–610.
15 J. L. STROMINGER, J. T. PARK, AND R. E. THOMPSON, *J. Biol. Chem.*, 234 (1959) 3263–3268.
16 H. BRADE, C. GALANOS, AND O. LÜDERITZ, *Eur. J. Biochem.*, 131 (1983) 195–200.
17 O. H. LOWRY, N. ROBERTS, K. LEINER, M. WU, AND L. FARR, *J. Biol. Chem.*, 207 (1954) 1–17.

18 Z. SYDORCZYK, W. KACA, H. BRADE, E. T. RIETSCHEL, V. SINNWELL, AND U. ZÄHRINGER, *Eur. J. Biochem.*, 168 (1987) 269–273.
19 K. BOCK AND C. PEDERSEN, *J. Chem. Soc., Perkin Trans. 2*, (1974) 293–297.
20 K. BOCK AND C. PEDERSEN, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 27–66.
21 M. SCHÜLLER, Thesis, Universität Hamburg, F.R.G., 1985.