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Preliminary communication

Structural studies of the *Vibrio cholerae* O-antigen

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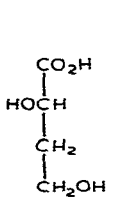
The lipopolysaccharide (LPS) from *Vibrio cholerae* has been studied by several groups[1]. It seems to contain lipid A, a core region, and O-specific side-chains, like LPS from other Gram-negative bacteria, but differs from these in some respects. Thus, it does not contain 3-deoxy-D-*manno*-octulosonic acid (KDO), which may be replaced by fructose[2]. Several sugars, including 2-amino-2,6-dideoxy-D-glucose[2] (quinovosamine), 4-amino-4-deoxy-L-arabinose, and 4-amino-4,6-dideoxy-D-mannose[3,4] (perosamine), have been found after acid hydrolysis, but no partial structures containing the corresponding sugars have been determined. We now report structural studies of the O-specific side-chains of the *V. cholerae* LPS.

The LPS from *V. cholerae*, serotype Inaba, was subjected to mild hydrolysis with acid[5], and a polysaccharide (PS) was isolated from the hydrolysate by gel filtration. The PS, $[\alpha]_D$ +16°, had $\bar{M}_w$ ~9000, as determined by gel filtration[6]. After hydrolysis with acid, glucose and a heptose were obtained; however, these sugars accounted for only a small part of the PS. The ^{13}C-n.m.r. spectrum of the PS (Table I) showed 10 strong signals and a number of weak signals, as expected for an O-antigen having a simple, regular structure, linked to a core.

Acid hydrolysis also released an acid that was identified as 3-deoxy-L-*glycero*-tetronic acid (*S*-2,4-dihydroxybutanoic acid; **1**) from the ^{13}C- (Table I) and ^{1}H-n.m.r.



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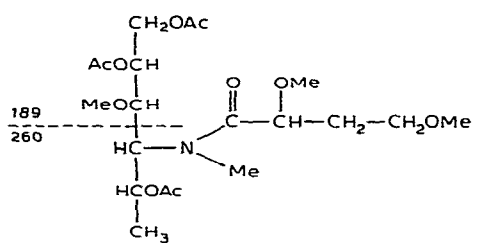

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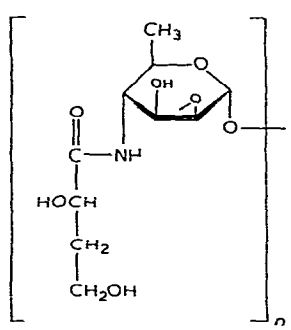

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spectra and the optical rotation of its sodium salt[7], $[\alpha]_D$ –8°. In the ^{1}H-n.m.r. spectrum of the sodium salt, signals were observed at δ 1.68–2.04 (m, 2 H, H-3), 3.68 (t, 2 H, J 9 Hz, H-4), and 4.0–4.2 (m, H-2), partly obscured by the HOD signal.

As also observed by Redmond[4], the PS is unusually resistant to acid hydrolysis. After acid hydrolysis, polymeric material ($[\alpha]_D$ +37°) remained and was purified by gel filtration. It was evident from the ^{13}C-n.m.r. spectrum (Table I) that *N*-deacylation had occurred and was essentially complete. Simultaneously, the core sugars were split off, and the weak signals assigned to these sugars are not present in the spectrum.

TABLE I

^{13}C-N.M.R. SHIFTS OF THE ORIGINAL AND MODIFIED *V. cholerae* PS AND SODIUM 3-DEOXY-L-*glycero*-TETRONATE

Carbon atom	*Chemical shift*		
	3-Deoxy-L-glycero-*tetronic acid (Na-salt)*	N-*Deacylated PS*[a]	*PS*[a]
C-1		102.5	102.7
C-2		78.7	79.2
C-3		68.3[b]	70.2[b]
C-4		56.3	55.1
C-5		67.9[b]	69.7[b]
C-6		18.8	18.8
C'-1	182.8		179.1
C'-2	71.0		71.4
C'-3	37.7		38.1
C'-4	59.8		60.1

[a] The spectrum was obtained at 85°. [b] Assignments which may be reversed

The PS could be hydrolysed with liquid hydrogen fluoride at room temperature, a reagent known to cleave glycosidic linkages but to leave amide linkages intact[8]. With the anhydrous reagent, glycosyl fluorides should be obtained. Our reagent was not perfectly dry, which may explain why reducing sugars were obtained in this reaction. In the present example, we obtained an anomeric mixture, $[\alpha]_D$ –7°, of perosamine *N*-acylated with 3-deoxy-L-*glycero*-tetronic acid. Similar treatment of the *N*-deacylated and *N*-acetylated PS yielded an anomeric mixture of *N*-acetylperosamine, $[\alpha]_D$ +20°.

Deamination of the *N*-deacylated PS with nitrous acid, followed by acid hydrolysis, yielded a mixture of rhamnose and 6-deoxyallose in the ratio 1·2.6. These products, which were tentatively identified by g.l.c.–m.s. of the alditol acetates (both retention times and spectra were indistinguishable from those given by authentic samples), are expected on deamination of 4-amino-4,6-dideoxy-D-*manno*-pyranosides[9].

The PS was methylated according to Hakomori[10] and hydrolysed with liquid hydrogen fluoride, and the product was reduced with sodium borohydride, acetylated, and analysed by g.l.c.–m.s. The mass spectrum of the main component (2) confirmed that it

was derived from a 4-amino-4,6-dideoxy sugar, *N*-acylated as discussed above. It was also transformed into the *N*-acetyl derivative, which gave the expected mass spectrum The methyl group at O-3 demonstrates that this position is free in the PS. This was confirmed by periodate-oxidation studies. As expected, the PS consumed only small amounts of periodate, arising from oxidation of the core sugars. The *N*-deacylated PS, however, consumed periodate with complete oxidation of the perosamine residues, demonstrating that these are linked through O-2 and not through O-3. The sugar residues in the PS should be α-linked, judging both from the optical rotation and the chemical shift (δ 5.1) of the anomeric proton in the ^{1}H-n.m.r. spectrum. In agreement with this finding, the C-1 signal in the ^{13}C-n.m.r. spectrum showed[11] $^{1}J_{C\text{-}H}$ 176 Hz.

From the foregoing data, it is proposed that the O-antigen of the *V. cholerae* serogroup Inaba LPS contains a homopolysaccharide composed of monomer residues having the structure 3. ^{13}C-N m.r. spectra of the corresponding PS prepared from classical, as well as biotype El Tor, *V. cholerae* serotype Ogawa indicate that they contain the same polymer chain. This polymer therefore probably represents the LPS antigen determinant (A) common to Inaba and Ogawa, a conclusion consistent with the results of hemagglutination-inhibition immunotests. The acid component, 3-deoxy-L-*glycero*-tetronic acid, has not been observed in Nature before. A homopolymer composed of 4-amino-4-deoxyhexose residues is also a new structural feature.

Structural studies of polysaccharides containing 4-amino-4-deoxy sugars are rendered difficult because of the lability of these sugars The methods used in the present investigation, hydrolysis in liquid hydrogen fluoride and deamination, may be of general value in studies of such polysaccharides.

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