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Note

Structure of the capsular polysaccharide and the O-side-chain of the lipopolysaccharide from *Acetobacter methanolicus* MB 135 (IMET 11402)

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As part of a systematic structural study of surface polysaccharides elaborated by *Acetobacter methanolicus*, we examined the capsular polysaccharide (CPS) and the O-side-chain of the lipopolysaccharide (LPS) from strain MB 135 (IMET 11402). Attention was focused on this strain because of its resistance against all known phages[1] of this species, and its opposition against chemical and physical trials of prophage induction[2].

Isolation of the CPS and LPS from lyophilised bacteria was conducted by the phenol–water extraction method[3]. The components were separated by ultracentrifugation, and the LPS was purified by DNase and RNase digestion followed by repeated ultracentrifugation steps. The CPS was obtained by DEAE-Sephacel ion-exchange chromatography[4] in the neutral effluent. The recoveries of the LPS and CPS were 2.2 and 15%, respectively.

Acid hydrolysis and identification of the products by sugar analyzer and by GLC as their derived alditol acetates afforded mannose as the only monosaccharide in the CPS, and as the predominant one in the LPS (Table I).

On the basis of the specific optical rotation, the configuration of mannose was determined to be D which was confirmed by calculation of the value for the CPS according to Klyne's rule[5] (Table II).

The ^{1}H NMR spectrum of the CPS contained signals for five anomeric protons at δ 5.04 ($J_{1,2}$ 1.8 Hz), 5.24 ($J_{1,2}$ 1.5 Hz), and 5.44 ppm ($J_{1,2}$ 1.5 Hz) (1, 3, and 1 H, respectively) indicating the D-mannosyl residues to be α-pyranoid[7].

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TABLE I

Monosaccharide composition[a] of the capsular polysaccharide (CPS) and the lipopolysaccharide (LPS) of *A. methanolicus* MB 135 (IMET 11402)

Component	Rha	Man	Gal	Glc	Hep [b]
CPS	1	98	0	1	0
LPS	10	85	1	2	1

[a] Molar proportions of sugar moieties obtained by GLC analysis are given. [b] L-*glycero*-D-*manno*-Heptose.

The ^{13}C NMR spectrum of the CPS (Fig. 1) contained signals for five anomeric carbons at 101.1, 101.8, and 103.2 ppm (1, 3, and 1 C, respectively), five unsubstituted hydroxymethyl groups at 62.4 ppm, and twenty other sugar carbons in the range 68–80 ppm. Thus, CPS is a regular mannan built up of pentasaccharide repeating units. The position of the signals for C-6 at 62.4 ppm is characteristic of the pyranose form[7]. The coupling constants $^1J_{C,H}$ 170–172 ppm for the five anomeric carbons were determined from the gated-decoupling spectrum of the CPS and proved all the anomeric carbons to be in α linkages[8–10].

On methylation analysis[11] of the CPS, only two partially methylated alditol acetates, namely 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methyl- and 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-mannitol, were obtained in the ratio 3.4:1 (by peak area), and characterised by GLC and GLC–MS[11]. Hence, the mannan is a nonbranched polymer with α-(1 → 2)- and α-(1 → 4)-glycosidic linkages.

Substitution of one of the mannose residues at position 4 followed also from the ^{13}C NMR spectrum which was interpreted using published data[8–10,12]. Four signals characteristic of unsubstituted C-4 were observed at 68.4 ppm, whereas the fifth C-4 signal was shifted downfield to 75.7 ppm due to the α-effect of glycosylation[9] (Fig. 1, Table III). Furthermore, the signal of the anomeric carbon at 103.2 ppm belonged to the 4-substituted mannosyl residue, whereas those of the 2-substituted residues resonated at higher field than 102 ppm, namely 101.8 and 101.1 ppm, due to the β-effect of glycosylation[8–10]. Only one signal for the unsubstituted[8,13] C-2

TABLE II

Calculation of the optical rotation of the CPS

	$[\alpha]_D$ (deg)	M_r	Molecular rotation (deg)
Methyl α-D-mannopyranoside [a]	+79.2	194	+153.6
CPS			
Calculated for 5 D-mannose residues	+95	810	+769
4 D-mannose and			
1 L-mannose residues	+57	810	+462
Experimental (*c* 5.0)	86.6		

[a] Ref 6.

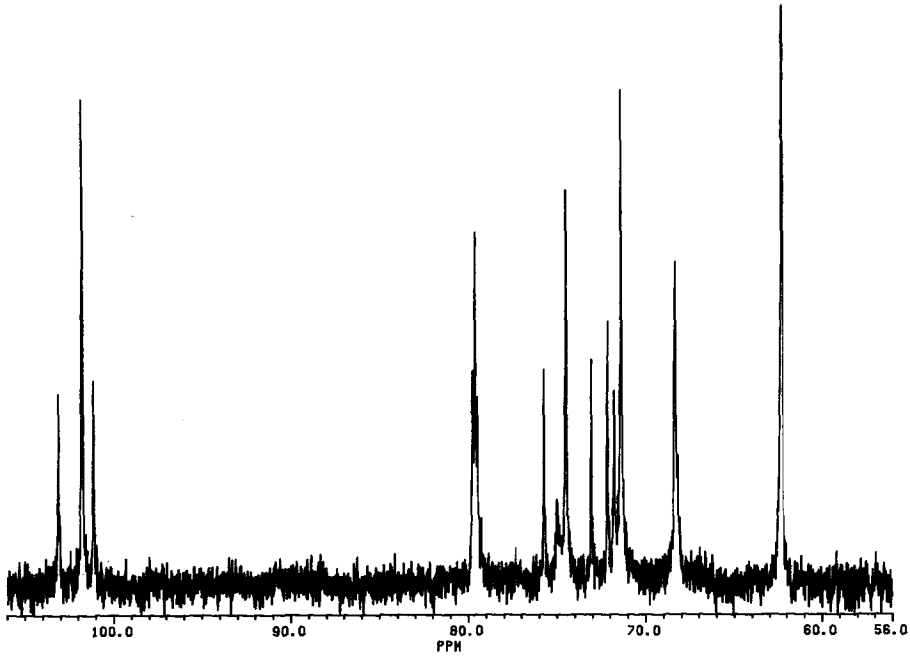

Fig. 1. ^{13}C NMR spectrum (62.89 MHz) of the capsular polysaccharide (CPS) of *Acetobacter methanolicus* MB 135 (IMET 11402).

(72.2 ppm) was observed, but four signals for the glycosylated[8,13] C-2 in the region 79.5 to 79.8 ppm.

Thus, the repeating unit of the CPS has the following structure.

→ 4)-α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 →
A **B** **C** **D** **E**

(1)

In order to determine the structure of the O-side-chain of the LPS, attempts to split the carbohydrate and lipid parts were carried out. As described in our previous paper[4], cleavage by acetic acid was not successful. Therefore, we used alkaline degradation (AD) to remove fatty acids. Saponification followed by

TABLE III

^{13}C NMR chemical shifts (δ, ppm) for the capsular polysaccharide of *Acetobacter methanolicus* MB135 (IMET 11402)

	C-1	C-2	C-3	C-4	C-5	C-6
→ 4)-α-Man*p*- **(A)**	103.2	72.2	71.8	75.7	73.1	62.4
→ 2)-α-Man*p*- **(B)**	101.8	79.8 [a]	71.4	68.4	74.5	62.4
→ 2)-α-Man*p*- **(C)**	101.8	79.6 [a]	71.4	68.4	74.5	62.4
→ 2)-α-Man*p*- **(D)**	101.8	79.6 [a]	71.4	68.4	74.5	62.4
→ 2)-α-Man*p*- **(E)**	101.1	79.5 [a]	71.4	68.4	75.0	62.4

[a] Assignments may be interchanged.

purification on Sephadex G-50 and Sephacryl S-300 resulted in a water-soluble polymeric material (LPS-AD). Its ^{13}C NMR spectrum contained all the signals for the mannan **1** shown above. Hence, as in other *A. methanolicus* strains[4], the CPS and the O-side-chain of the LPS are built up of the same repeating units. Other components listed in Table I most likely enter into the core constituent of the LPS.

Mannans, including those of pentasaccharide repeating units, were described as O-antigens for some enterobacteria[14], but none is identical to structure **1**.

EXPERIMENTAL

Bacterial strain, growth conditions, and preparation of polysaccharides.—*A. methanolicus* MB135 (IMET 11402) was grown and the cells were processed as described[4]. Starting from 20 g of lyophilised bacteria, 440 mg (2.2%) of LPS and 2.95 g (15%) of CPS were recovered.

General methods.—NMR spectra (^1H and ^{13}C) were recorded for samples in D_2O with Bruker WM-250 and AM-300 spectrometers at 70 and 30°C, respectively.

The optical rotation was measured on an aqueous solution with a Jasco Model DIP 360 digital polarimeter at 25°C.

GLC-MS was performed on a Varian MAT 311 instrument.

Degradation of the LPS, acid hydrolyses, sugar analyses, and gel-permeation chromatography were described earlier[4].

Methylation analysis was carried out by standard procedures[11], and the products were identified by GLC–MS.

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