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Structure of the *Acetobacter methanolicus* MB 129 capsular polysaccharide, and of oligosaccharides resulting from degradation by bacteriophage *Acm7*

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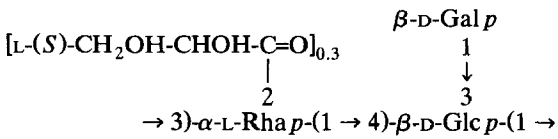
Abstract

The capsular polysaccharide of *Acetobacter methanolicus* MB 129 consists of D-Glc, D-Gal, L-Rha, and L-glyceric acid in the molar ratios 1:1:1:0.3. Periodate oxidation, methylation analysis, solvolysis with HF, and detailed ^{1}H and ^{13}C NMR analysis resulted in the structure of the repeating unit shown below.



Bacteriophage *Acm7*-associated endo-α-L-rhamnopyranoside hydrolase depolymerizes the CPS even in the presence of the *O*-acyl group, to give the respective hexa-, nona-, and dodeca-saccharides.

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1. Introduction

In previous investigations [1–4], we described the structures of the capsular polysaccharides (CPS) and the O-side-chains of the lipopolysaccharides (LPS) of four *Acetobacter methanolicus* strains. A peculiar feature is the structural identity of the repeating units of the CPS and O-side-chain of the LPS in each of the strains. For two of those strains, lytic bacteriophages are known [5] which use both CPS and LPS as receptors [1,3].

A. methanolicus MB 129 was isolated from a strain MB 70 batch after infection by the bacteriophage *Acm*5. Lysogenization resulted in a derivative which was resistant to infection by *Acm*5 [6]. On the other hand, this strain became sensitive to the action of bacteriophage *Acm*7 [5,6]. We report here on the structure of the CPS of the altered phenotype, and on the structures of oligosaccharides which were obtained by chemical and phage *Acm*7-mediated degradation of the CPS.

2. Results and discussion

The CPS was isolated from lyophilized bacteria by the phenol–water method [7] followed by sedimentation of the LPS by ultracentrifugation, and ion-exchange chromatography of the clear supernatant solution. The major polysaccharide component (CPS, 72%) was recovered from the neutral eluate.

Acid hydrolysis of the CPS and analysis with a sugar analyzer and by GLC of the respective alditol acetates revealed equal amounts of Rha, Gal, and Glc. Enzymatic oxidation of the hydrolysate with D-galactose oxidase [3,8] and D-glucose oxidase [9] proved the D configuration for both galactose and glucose.

The ^{13}C NMR spectrum of the native CPS (Fig. 1A) contained signals for anomeric carbons at 104.8, 103.9, 100.1, and 97.2 ppm (Table 1) with the ratios 1:1:0.7:0.3, respectively, and signals for one carbonyl group at 174.1 ppm, one C-6 of Rha at 17.8 ppm, and other carbons in the region 61–81 ppm represented by 20 signals of different intensities. After saponification of the CPS with aqueous ammonia, followed by dialysis, the signal (Fig. 1, Table 1) for the carbonyl group, those at 76.3 and 64.4 ppm, and the signals of minor intensities at 97.2, 78.7, and 74.0 ppm disappeared. On the other hand, signals at 100.1, 80.9, and 71.2 ppm were enhanced. Each of the three pairs of signals (104.7, 104.8; 79.8, 79.9; and 61.2, 61.5 ppm) collapsed to single signals. These data clearly demonstrate the presence of an alkali-labile substituent attached to a specific position of the polysaccharide.

The ^{13}C NMR spectrum (Fig. 1B) of the alkali-degraded polymer (CPS-AD) contains three signals for the anomeric carbons, 14 signals in the region 61–82 ppm, and one signal at 17.8 ppm, indicating a regular polysaccharide with a trisaccharide repeating unit built up of Glc, Gal, and Rha.

Methylation analysis [10] of the *O*-deacylated polymer (CPS-AD) led to the identification of 1,3,5-tri-*O*-acetyl-2,4-di-*O*-methylrhamnitol, 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol, and 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methylhexitol.

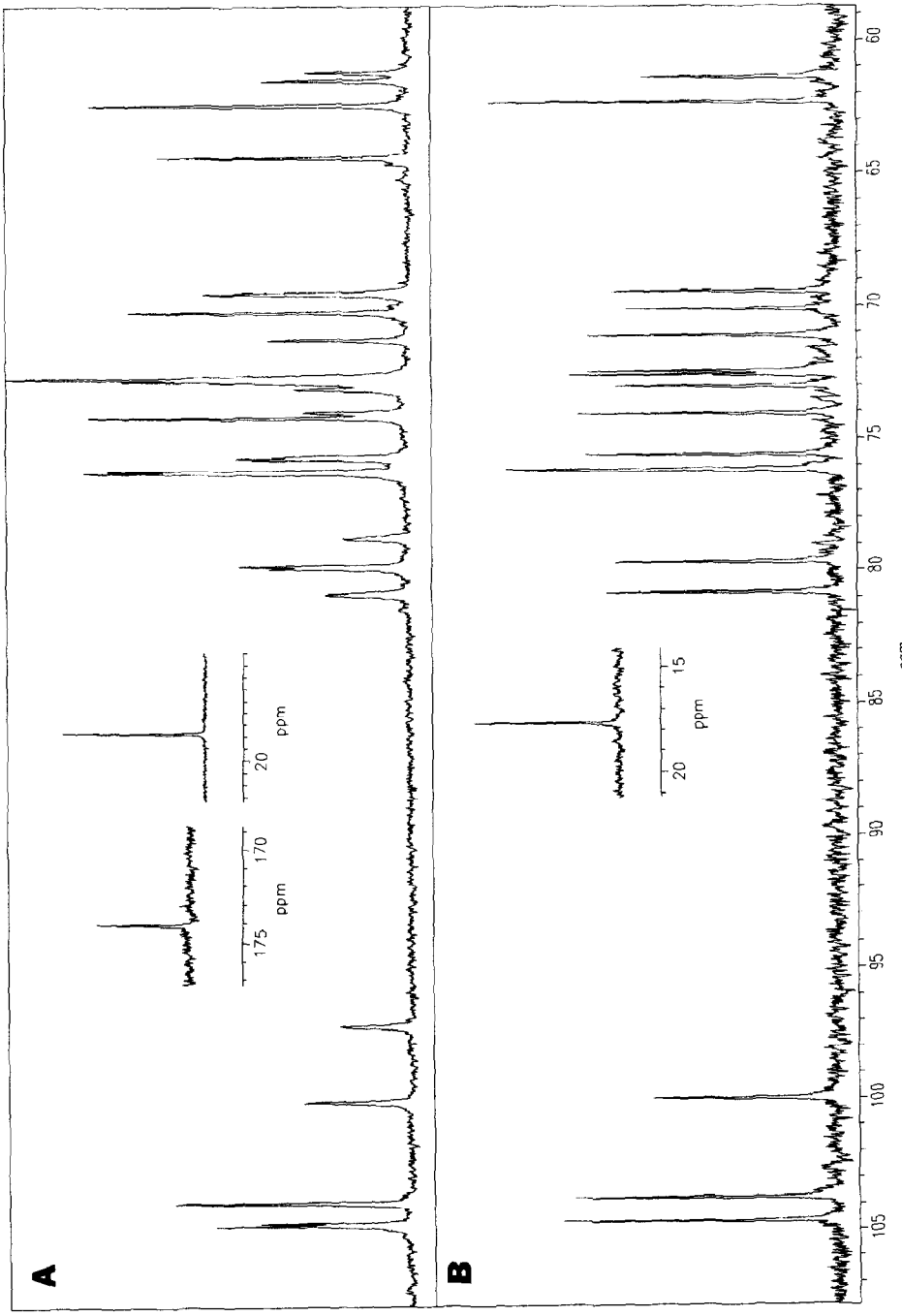


Fig. 1. ^{13}C NMR spectra of the capsular polysaccharide (CPS) (A) and the alkali-degraded CPS (CPS-AD) (B) of *A. methanolicus* MB 129.

Table 1
^{13}C NMR spectra data (δ, ppm) for the CPS and the *O*-deacylated CPS of *A. methanolicus* MB 129.

Residue	C-1	C-2	C-3	C-4	C-5	C-6
CPS (2) [a]						
→ 4)-β-D-Glc*p*-(1 →	104.8	74.2	79.9	73.1	76.3	61.5
3 ↑						
	104.7	74.2	79.8	72.9	76.3	61.2
→ 3)-α-L-Rha*p*-(1 →	100.1	71.2	80.9	72.7	69.5	17.8
→ 3)-α-L-Rha*p*-(1 →	97.2	74.0	78.7	72.9	69.5	17.8
2 ↑						
L-Glycerate	174.1	76.3	64.4			
β-D-Gal*p*-(1 →	103.9	72.7	75.7	70.2	76.2	62.4
O-Deacylated CPS (CPS-AD)						
→ 4)-β-D-Glc*p*-(1 →	104.8	74.2	79.8	73.1	76.3	61.5
3 ↑						
→ 3)-α-L-Rha*p*-(1 →	100.1	71.2	80.9	72.6	69.5	17.8
β-D-Gal*p*-(1 →	103.9	72.7	75.8	70.2	76.2	62.4

[a] The signals of Glc split into two series due to the partial acylation of the rhamnose moiety.

Hence, the CPS-AD repeating unit consists of a terminal and a disubstituted hexose residue, and 3-substituted Rha.

Periodate oxidation of the CPS followed by borohydride reduction, hydrolysis, and gel permeation chromatography gave a disaccharide (OS-1), a tetrasaccharide (OS-2), and a polymeric product (CPS-SD), all being built up of Rha and Glc. The ^{13}C NMR spectrum of CPS-SD was identical to that of the glucorhamnan previously identified [11] as the exopolysaccharide of the type strain of *A. methanolicus* MB 58/4 (Table 2), corresponding to structure **1**.

→ 3)-α-L-Rha*p*-(1 → 4)-β-D-Glc*p*-(1 →

1

OS-1 and OS-2 were identified by ^{13}C NMR analysis as degradation products of polymer **1**, with Rha at the reducing end (Table 2), obtained at the hydrolysis step. Assignments of the spectra were made on the basis of the published data for 3-*O*-β-D-glucopyranosyl-L-rhamnopyranose [12], a glycosylated L-rhamnitol [13], and L-rhamno-D-glucan [11].

On the basis of the results obtained by methylation analysis and Smith degradation, it was concluded that Gal represented the lateral residue attached to Glc at position 3.

The anomeric configurations of the sugar units in the CPS were established from the gated-decoupled ^{13}C NMR spectrum. The signals of C-1 at 104.8 and 103.9 ppm had relatively small $^{1}J_{C,H}$ values (163.6 and 161.1 Hz, respectively), indicating β-glycosidic linkages [14,15]. The relatively large coupling constants $^{1}J_{C,H}$ 173.3 and 170.1 Hz for the signals at 100.1 and 97.3 ppm, respectively, showed that the corresponding sugar residues were α-linked [14,15].

The anomeric configurations for the pyranoses having *gluco* and *galacto*

Table 2
^{13}C NMR spectral data (δ, ppm) of the Smith-degradation products OS-1, OS-2, OS-2-ol [a], and CPS-SD from *A. methanolicus* MB 129

Residue	C-1	C-2	C-3	C-4	C-5	C-6
OS-1						
β-D-Glc*p*-(1 →	104.9	74.6	76.8	70.7	77.0	61.8
→ 3)-α-L-Rha*p*	94.9	71.8	81.0	72.4	69.4	17.9
→ 3)-β-L-Rha*p*	94.5	72.1	83.4	72.1	72.9	17.9
OS-2						
β-D-Glc*p*-(1 →	105.1	74.8	77.0	70.9	77.1	62.0
→ 3)-α-L-Rha*p*-(1 →	102.0	71.6	78.6	72.6	70.4	17.9
→ 4)-β-D-Glc*p*-(1 →	105.0	75.7	75.1	78.6	76.3	61.6
→ 3)-α-L-Rha*p*	95.1	71.9	81.1	72.6	69.5	18.1
→ 3)-β-L-Rha*p*	94.6	72.3	83.6	72.3	73.1	17.9
OS-2-ol [a]						
β-D-Glc*p*-(1 →	105.2	74.9	77.1	71.0	77.2	62.1
→ 3)-α-L-Rha*p*-(1 →	102.0	71.6	81.4	72.8	70.5	17.9
→ 4)-β-D-Glc*p*-(1 →	104.5	75.8	75.9	79.0	76.3	61.9
→ 3)-L-Rha-ol	64.1	72.4	79.3	75.3	67.7	20.5
CPS-SD (1)						
→ 4)-β-D-Glc*p*-(1 →	104.8	75.0	75.6	78.5	76.1	61.5
Ref 11	104.7	75.1	75.6	79.3	76.2	61.7
→ 3)-α-L-Rha*p*-(1 →	101.8	71.5	81.4	72.2	70.3	17.8
Ref 11	101.6	71.6	80.8	72.4	70.4	17.9

[a] Borohydride-reduced OS-2.

configurations were also deduced [16] from the ^1H NMR spectrum of CPS-AD, which contained two doublets for the anomeric protons at 4.86 and 4.69 ppm with $J_{1,2}$ 7.8 and 8.0 Hz, respectively, indicating the β-glycosidic linkages of D-Gal*p* and D-Glc*p* (Fig. 2). The H-1 of rhamnose gave a narrow doublet at 4.94 ppm, $J_{1,2}$ 2.0 Hz (Fig. 2), which corresponded [16] to the α-glycosidic linkage (Fig. 1).

In order to assign the ^1H NMR spectrum of CPS-AD (Table 3), the ^1H, ^1H COSY (Fig. 3) and relayed ^1H, ^1H COSY (RCT-1) (not shown) spectra were recorded. The signals of α-L-Rha were identified from the ^1H NMR spectrum (Fig. 2, Table 3), using the known data of chemical shifts [17–19], the coupling constants [16,19], and the connectivities in the ^1H, ^1H COSY spectrum. The signals of the β-D-Glc residue were identified from the ^1H, ^1H COSY spectrum, using the cross-peaks H-1/H-2 (4.69, 3.64 ppm), H-2/H-3 (3.64, 3.99 ppm), and H-3/H-4 (3.99, 3.80 ppm), and the coupling constants $J_{2,3} \approx J_{3,4} \approx 9.5$ Hz. The position of the signal of H-5 was obtained from both ^1H, ^1H COSY and RCT-1 spectra (H-3/H-5 at 3.99, 3.50 ppm, and H-4/H-5 at 3.80, 3.50 ppm), and those for H-6a and H-6b from the ^1H, ^1H COSY spectrum, using the respective cross-peaks with H-5.

The signals of the β-D-Gal residue were assigned in a similar manner (Figs. 2 and 3). However, the coupling constants for H-5 could not be estimated because of overlapping of a number of signals for Glc and Gal.

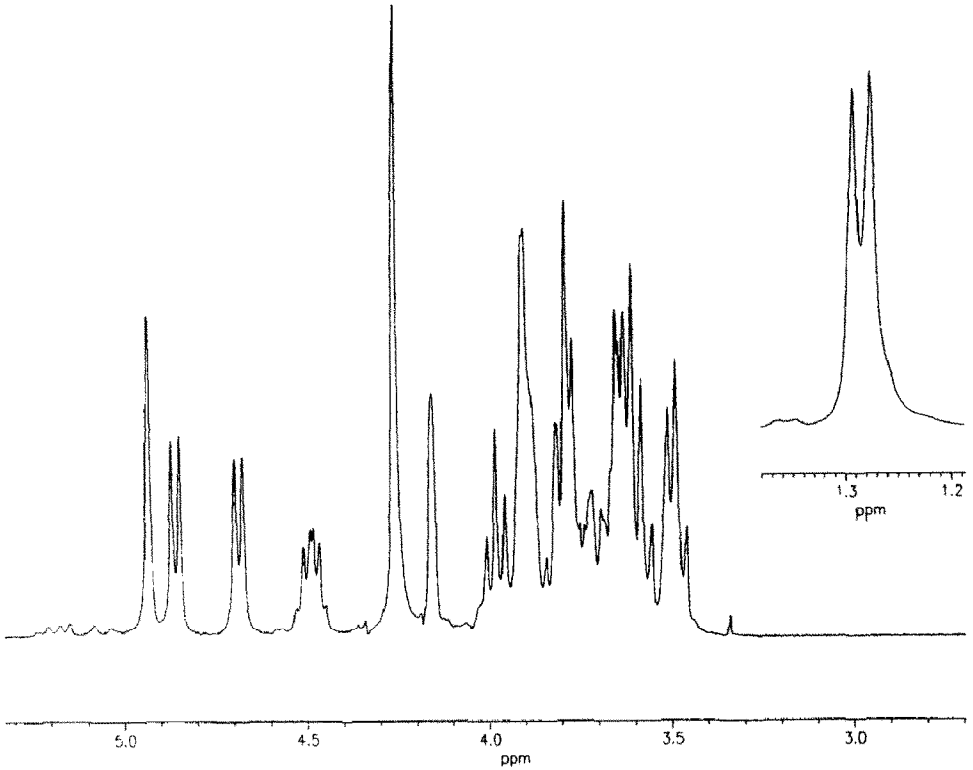


Fig. 2. ^{1}H NMR spectrum of the alkali-degraded capsular polysaccharide (CPS-AD) of *A. methanolicus* MB 129.

The ^{13}C NMR spectrum of the CPS-AD (Table 1) was unambiguously assigned using the ^{1}H, ^{13}C heteronuclear correlation (HETCOR) spectrum (Fig. 4).

The ^{13}C NMR spectrum (Fig. 1A) of the CPS contained pairs of signals at 100.1 and 97.2 ppm, 71.2 and 74.0 ppm, and 80.9 and 78.7 ppm for C-1, C-2, and C-3 of the Rha residue. For each pair of signals, the ratio of intensities was $\sim 2:1$. In the spectrum of CPS-AD, the signals for C-1, C-2, and C-3 of Rha were not split, and the signals at 64.4, 76.3, and 174.1 ppm of the CPS could not be seen; all signals in this spectrum had the same intensity. Hence, in the CPS, Rha is partially substituted by an alkali-labile component containing one carbonyl group (174.1 ppm), and two carbons with chemical shifts typical of a CHOH (76.3 ppm) and a CH_2OH (64.8 ppm) group (Fig. 1, Table 1). The position of the *O*-acyl substituent was determined from the ^{13}C NMR spectrum (Fig. 1, Table 1), using the rules on acylation effects [20]. The signal of C-2 of Rha was shifted downfield by 2.8 ppm, whereas those of C-1 and C-3 were shifted upfield by 2.9 and 2.2 ppm, respectively. Hence, the acyl substituent is linked to position 2 of the Rha residue in nearly one-third of the repeating units, as follows from the signal intensity ratio. Such a partial acylation also caused smaller effects on C-1, C-2, C-3, and C-6

Table 3
^{1}H NMR spectral data of the *O*-deacylated CPS of *A. methanolicus* MB 129

Residue	Proton	Chemical shift (ppm)	Multiplicity	Coupling constant (Hz)	
→ 4)-β-D-Glc*p*-(1 →	H-1	4.69	d	$J_{1,2}$	8.0
3	H-2	3.64	dd	$J_{2,3}$	9.5
↑	H-3	3.99	dd	$J_{3,4}$	9.5
	H-4	3.80	dd	$J_{4,5}$	9.3
	H-5	3.50	ddd	$J_{5,6b}$	~4
	H-6a	3.84	dd	$J_{5,6a}$	2.4
	H-6b	3.72	dd	$J_{6a,6b}$	11.8
→ 3)-α-L-Rha*p*-(1 →	H-1	4.94	d	$J_{1,2}$	2.0
	H-2	4.16	dd	$J_{2,3}$	3.2
	H-3	3.89	dd	$J_{3,4}$	9.6
	H-4	3.59	dd	$J_{4,5}$	9.6
	H-5	4.49	dq	$J_{5,6}$	6.1
	H-6	1.28	d		
β-D-Gal*p*-(1 →	H-1	4.86	d	$J_{1,2}$	7.8
	H-2	3.49	dd	$J_{2,3}$	9.6
	H-3	3.64	dd	$J_{3,4}$	3.2
	H-4	3.91	dd	$J_{4,5}$	≤1
	H-5	3.66	ddd		
	H-6a,b	~3.80	m		

(−0.1, −0.2, −0.2, and −0.3 ppm, respectively) of the Glc residue linked to C-3 of the Rha (Fig. 1, Table 1).

The acyl substituent was identified as follows. On chromatography on Fractogel TSK HW-40(S) of the products of saponification of the CPS together with CPS-AD (void volume peak), a low molecular weight fraction was isolated. In the ^{1}H NMR spectrum of this substance, three signals for protons were observed. Two of them (δ 2.99 ppm, $J_{2,3b}$ 3.9 and $J_{3a,3b}$ 9.8 Hz; and δ 3.03 ppm, $J_{2,3a}$ 3.1 and $J_{3a,3b}$ 9.8 Hz) belong to a CH_2OH group, and the third one (δ 3.37 ppm, $J_{2,3a}$ 3.1 and $J_{2,3b}$ 3.9 Hz) originate from a CHOH group, the three protons of both groups coupling to each other with the typical dd signal multiplicities. The ^{13}C NMR spectrum contained the signals of a carbonyl (δ 178.3 ppm), a CHOH (73.7 ppm), and a CH_2OH group (δ 65.2 ppm). Thus, the acyl substituent was identified as glyceric acid. The negative optical rotation of the ammonium salt {$[\alpha]_D$ −8.3° (*c* 0.8, H_2O)} proved it to possess the L-(*S*) configuration (cf. the published data [21] + 14.4° and −14.6° for the Ca salts of D- and L-glyceric acid, respectively) *.

On the basis of the results discussed above, it could be concluded that the capsular polysaccharide from *A. methanolicus* MB 129 is built up of trisaccharide repeating units **2**.

* This assignment was confirmed by the isolation of methyl L-glycerate, $[\alpha]_D$ −12.7° (*c* 0.6, H_2O), after acidic methanolysis of native CPS; lit. [21] $[\alpha]_D$ −14.6° (H_2O).

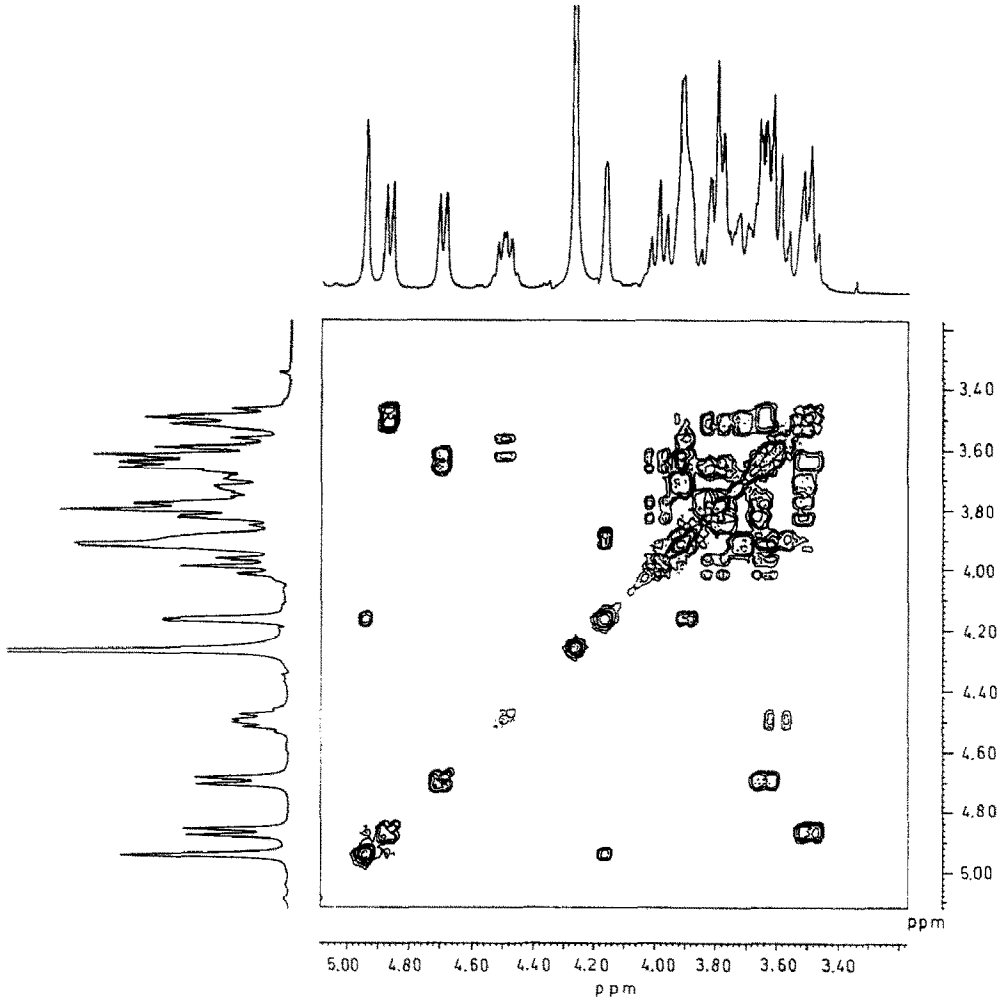
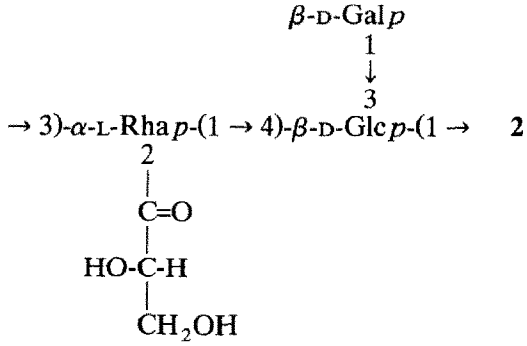


Fig. 3. ^{1}H, ^{1}H COSY spectrum of the alkali-degraded capsular polysaccharide (CPS-AD) of *A. methanolicus* MB 129.



Solvolysis of the CPS with HF [22] at −70° C, followed by borohydride reduction, and separation of the products by gel permeation chromatography on Fractogel TSK HW-40(S) gave a tri-, a hexa-, and a nona-saccharide (OS-3, OS-4, and OS-5, respectively), all containing Glc, Gal, and Rha as determined by GLC of their alditol acetates. The structures of OS-3 and OS-4 were determined (Table 4) on the basis of chemical shifts in the ^{13}C NMR spectra, using the data for the CPS-AD (Table 1), OS-1, OS-2, and OS-2-ol (Table II), and literature data [12,13,17]. OS-5 was supposed to be an oligomer homologue of OS-3 and OS-4 composed of three repeating units. Thus, solvolysis with HF cleaved some rhamnopyranosyl linkages and removed the acyl substituent. The resulting oligosaccharides confirmed the structure of the CPS and were used as model compounds for identification of the products obtained from CPS–virus interaction as described below.

β-D-Gal*p*-(1 → 3)-β-D-Glc*p*-(1 → 3)-L-Rha-ol OS-3

C″ **A″** **B″**

C
β-D-Gal*p*
1
↓
3

β-D-Gal*p*-(1 → 3)-β-D-Glc*p*-(1-[→ 3)-L-Rha*p*-(1 → 4)-β-D-Glc*p*-(1-]$_n$ → 3)-L-Rha-ol

C″ **A″** **B** **A** **B″**

$n = 1$ OS-4
$n = 2$ OS-5
$n = 3$ OS-6

In order to investigate the mode of action of the bacteriophage *Acm*7 on the CPS component of its host [5,6], CPS **2**, deacylated CPS (CPS-AD), and Smith-degraded CPS **1** (CPS-SD) were exposed to the virus (assays 1, 2, and 3, respectively).

Assay 1 gave oligosaccharides eluted from Fractogel TSK HW-40(S) near the position of hexa- and nona-saccharides, and higher saccharides up to the void volume. The hexa- and nona-saccharide fractions, according to the ^{13}C NMR data, were heterogeneous due to partial substitution by L-glyceric acid. After borohydride reduction, homogeneous oligosaccharides were obtained and identified by ^{13}C NMR analyses as OS-4 and OS-5 (Table 4). Furthermore, the virus-depolymerase produced a dodecasaccharide which, after borohydride reduction, was spectroscopically characterized (Table 4) as an assembly of four repeating units (OS-6).

L-Glyceric acid was absent in all cases from the reduced oligosaccharides, because of borohydride reduction and/or alkaline conditions during this treatment.

Assay 2 provided pure hexa-, nona-, and dodeca-saccharides, which were converted by borohydride reduction into OS-4, OS-5, and OS-6 (Table 4).

Assay 3 resulted only in the polymeric starting material, eluted with the void volume on TSK HW-40(S), which as judged by a photometric assay [23] had no detectable reducing groups.

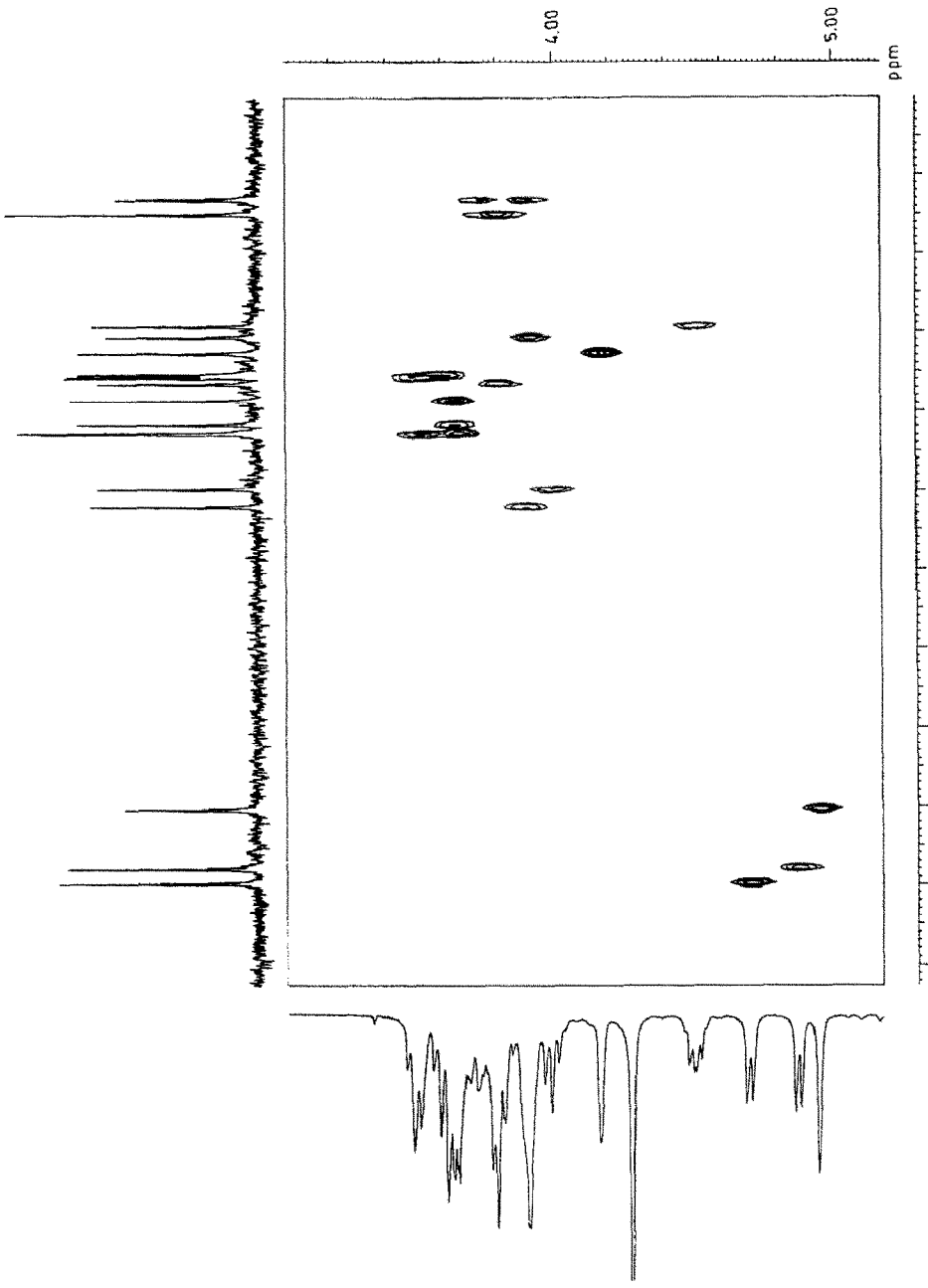


Fig. 4. ^{1}H, ^{13}C Heteronuclear correlation spectrum of the alkali-degraded capsular polysaccharide (CPS-AD) of *A. methanolicus* MB 129.

Table 4
^{13}C NMR spectral data (δ, ppm) of the oligosaccharides obtained by hydrogen fluoride solvolysis of the CPS, and those formed from the CPS and CDS-AD by depolymerisation with the bacteriophage *Acm*7, and subsequent borohydride reduction

Residue	C-1	C-2	C-3	C-4	C-5	C-6
Trisaccharide (OS-3)						
Residue A″	104.8	74.7	85.8	69.9	76.8	62.3
Residue B″	64.1	72.7	79.1	75.9	67.5	20.7
Residue C″	104.4	72.6	74.1	70.1	76.8	62.5
Hexasaccharide (OS-4)						
Residue A″	104.8	74.5	86.1	69.7	77.0	62.1
Residue B	100.6	71.4	81.5	72.9 [a]	69.9	18.1
Residue A	105.0	74.5	80.3	73.2 [a]	76.6	61.9
Residue B″	64.3	72.8	79.8	76.1	67.9	20.5
Residue C″	104.6	73.9	76.1	70.2	76.8	62.5
Residue C	104.2	73.0 [a]	76.1	70.5	76.6	62.7
Nonasaccharide (OS-5)						
Residue A″	104.9	74.2	85.3	69.2	76.5	61.7
Residue B	100.0	70.9	81.1	72.6 [a]	69.5	18.1
Residue A	105.1	73.9	79.5	72.4 [a]	76.5 [b]	61.0
Residue B″	63.9	73.7	78.7	75.8	67.1	20.5
Residue C″	104.4	72.8	75.6	70.2	76.5	62.2
Residue C	104.0	72.4 [a]	75.6	70.2	76.3 [b]	62.4
Dodecasaccharide (OS-6)						
Residue A″	105.1	74.5	85.5	69.7	76.7	62.4
Residue B	100.2	71.4	81.3	72.8 [a]	69.9	18.1
Residue A	105.3	74.5	79.6	72.8 [a]	76.7 [b]	61.8
Residue B″	64.3	73.6	79.6	76.2	67.9	20.5
Residue C″	104.7	74.3	76.2	70.5	76.7	62.4
Residue C	104.2	72.6 [a]	76.2 [b]	70.5	76.5 [b]	62.7

[a,b] Signals may be interchanged.

Hence, the native CPS represents the primary cell-surface receptor for the bacteriophage *Acm*7. Acylation by L-glyceric acid has, according to our experiments, no influence on this activity. However, if the β-D-Gal*p* moiety is missing, the phage-depolymerase is unable to split the polymer. This implies that the branching sugar is essential for the enzyme recognition site.

The viral depolymerase of *Acm*7, like those studied previously [1,3], acts as a hydrolase, unlike lyases which form the corresponding elimination products [25]. Moreover, the size of the oligosaccharides formed and the absence of the trisaccharide point to an extended catalytic site, and an endo-mechanism for the viral [24,25] α-L-rhamnopyranoside hydrolase.

A. methanolicus MB129 expresses a CPS with the same glucorhamnan backbone as found for the extracellular polysaccharide [11] of *A. methanolicus* MB58/4, but additionally possesses β-D-Gal and L-glyceroyl residues as substituents. The α-glucan and β-galactan components, which were identified [3] as the CPS and

O-side-chain of the LPS of the parent strain [6], were not found in *A. methanolicus* MB129.

In a following paper, we will describe the structure of the O-side-chain of the LPS which, in contrast to the other strains of this genus, differs from that of the corresponding CPS.

3. Experimental

Bacterial strain, growth conditions, and preparation of polysaccharides.—*A. methanolicus* MB129 was grown and the cells were processed as described earlier [1]. Starting from 25 g of lyophilized bacteria, 2.2 g (9%) of CPS and 0.35 g (1.4%) of LPS were isolated using the phenol–water extraction method [7]. The purification of CPS was described earlier [1].

General methods.—The ^{1}H and ^{13}C NMR spectra were recorded for samples in D_2O with Bruker WM-250 and AM-300 spectrometers at 30 and 60° C for oligo- and polysaccharides, respectively.

Optical rotations were measured for aqueous solutions with a Jasco Model DIP 360 digital polarimeter at 25° C.

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

Monosaccharides were released from oligo- or poly-saccharides (1–2 mg) by acid hydrolyses (M HCl, 1 mL, 105° C, 6–8 h) in sealed tubes. The products were analyzed with a Technicon Sugar Analyzer and by GLC of the derived alditol acetates [3,26].

Reducing sugars were determined spectrophotometrically, using a modification [23] of the Somogyi method.

The absolute configurations of Glc and Gal were determined enzymatically on aliquots of the acid hydrolysate, using D-glucose oxidase [9] (EC 1.1.3.4) (Boehringer) and D-galactose oxidase [8] (EC 1.1.3.9) (Sigma) as described previously [3].

Gel-permeation chromatography was conducted on columns of Fractogel TSK HW-40(S) (80 × 1.6 cm; 50 × 1.6 cm) and Sephadex G-50 (70 × 3.0 cm). Eluate was monitored for carbohydrates, using a differential refractometer (Knauer).

Methylation analysis was carried out by the standard procedure [10], and the products were identified by GLC–MS.

Reduction of oligosaccharides was performed with a solution of $NaBH_4$ in water (10 mg/mL, 1 mL for 5–10 mg of OS). Residual borohydride was destroyed with 10% AcOH, cations were removed using Dowex 50W X8 (H^+) resin, and several evaporation steps with methanol were applied to eliminate borate.

O-Deacylation.—CPS (100 mg) was dissolved in 7 M NH_4OH (15 mL) and stirred at room temperature for 18 h. The solvent was removed by evaporation, and the sample was either dialyzed extensively against water or applied to Sephadex G-50, to yield the polymeric material (78 and 85 mg, respectively) and a low molecular weight fraction. The latter was further purified by gel permeation chromatography on Fractogel TSK HW-40(S) to give the NH_4-salt of L-glyceric acid (8 mg).

Smith degradation.—CPS (120 mg) was treated with 50 mM sodium periodate in 50 mM AcOH (25 mL) for 24 h at 4° C. The reaction was stopped by the addition of ethylene glycol (0.5 mL) and, after dialysis against water, the retentate was concentrated to a small volume (2–3 mL), reduced with $NaBH_4$ (50 mg), dialyzed, and lyophilized to yield CPS-SD (84 mg). Hydrolysis was carried out with 0.1 M HCl (4 mL) at 100° C for 30 min, the mixture was freeze-dried, and the products were separated on Fractogel TSK HW-40(S).

Solvolysis with anhydrous hydrogen fluoride.—CPS (60 mg) was treated in liquid HF (~ 10 mL) at −70° C for 60 min, the products were precipitated with diethyl ether, and the HF was removed by repeated washing steps. The oligosaccharides were separated on Fractogel TSK HW-40(S) to give a trisaccharide (35 mg), a hexasaccharide (12 mg), and a nonasaccharide (2 mg).

Virus-catalyzed depolymerization of the bacterial polysaccharides.—CPS (25 mg, assay 1), CPS-AD (25 mg, assay 2), and CPS-SD − (10 mg, assay 3) were incubated with bacteriophage *Acm*7 (3×10^{11} PFU for the assays 1 and 2; 10^{11} PFU for assay 3) in 50 mM NaOAc buffer, pH 4.5, containing 50-mM NaCl, 5×10^{-4} M $CaCl_2$, and 5×10^{-4} M $MgCl_2$ (10 mg/mL) at 28° C for 3 h. Assay mixtures were concentrated to ca. 1 mL and fractionated on Fractogel TSK HW-40(S). Major products were collected, lyophilized, reduced with $NaBH_4$, and purified by gel permeation chromatography on TSK HW-40(S).

From assay 1, OS-4 (3.4 mg) and OS-5 (5.1 mg) were obtained. Assay 2 provided OS-4 (3.4 mg), OS-5 (3.6 mg), and OS-6 (4.4 mg). Assay 3 yielded only the polymeric undegraded starting material.

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