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Note

Structure of the O-specific polysaccharide of *Citrobacter freundii* O3a,3b,1c

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Abstract

The following structure of the O-specific polysaccharide of *Citrobacter freundii* O3a,3b,1c containing D-mannose and D-rhamnose was established using sugar analysis and NMR spectroscopy, including computer-assisted analysis of the ^{13}C NMR spectrum, 2D COSY, H,H-relayed COSY, heteronuclear ^{13}C, ^{1}H correlation (HETCOR), and rotating-frame NOE spectroscopy (ROESY): → 4)-α-D-Man*p*-(1 → 3)-β-D-Rha*p*-(1 → 4)-β-D-Rha*p*-(1 → .

Keywords: Lipopolysaccharide; O-specific polysaccharide; D-Rhamnose; *Citrobacter freundii*

Citrobacter freundii is an enterobacterium taxonomically related to *Salmonella*. Strains of *C. freundii* are serologically heterogeneous due to structural diversity of their surface lipopolysaccharide (O-antigen) [1]. Structural and serological studies showed that in some serogroups the O-specific polysaccharide chains of the lipopolysaccharides are similar to those of *Salmonella* [1–3], while others have unique structures (Ref. [4] and references cited therein). Now, we report on the structure of the O-specific polysaccharide of *C. freundii* serogroup O3a,3b,1c.

The O-specific polysaccharide was obtained by degradation of the lipopolysaccharide, isolated from dry bacterial cells by the phenol–water procedure [5], followed by GPC on Sephadex G-50. Sugar analysis, including determination of the absolute configurations [6], showed that the polysaccharide contains D-mannose and D-rhamnose in the ratio ~ 1:2.

The ^{13}C NMR spectrum (Fig. 1) of the polysaccharide contained signals for three anomeric carbons at δ 100.4, 101.5, and 101.8, two CH_3–C groups (C-6 of Rha) at δ 18.1 (2 C), one unsubstituted $HOCH_2$–C group (C-6 of Man) at δ 62.2, and 12 sugar ring carbons in the region δ 71.1–83.6. Accordingly, the ^{1}H NMR spectrum of the polysaccharide contained, inter alia, signals for three anomeric protons at δ 4.61, 4.64, and 5.03 (all broadened singlets), and two CH_3–C groups (H-6 of Rha) at δ 1.25 (6 H, d, $J_{5,6}$ 6 Hz).

These data showed that the polysaccharide is linear

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Table 1
75 MHz ^{13}C NMR data for the O-specific polysaccharide (δ in ppm). Data calculated by the published method [7,8] are given in parenthesis

	C-1	C-2	C-3	C-4	C-5	C-6
→ 4)-α-D-Man*p*-(1 →	100.4 (103.6)	71.1 (71.5)	70.7 (70.5)	78.4 (78.5)	73.3 (73.2)	62.2 (61.6)
→ 3)-β-D-Rha*p*^{II}-(1 →	101.8 (101.4)	71.8 (71.4)	81.9 (81.0)	72.5 (72.5)	73.6 (73.2)	18.1 (18.0)
→ 4)-β-D-Rha*p*^{I}-(1 →	101.5 (101.4)	71.4 (71.6)	72.9 (73.0)	83.6 (83.4)	72.4 (72.2)	18.1 (18.0)

and has a trisaccharide repeating unit containing one residue of D-mannose and two residues of D-rhamnose.

Computer-assisted analysis [7,8] revealed only one linear structure (**1**) that fitted with the ^{13}C NMR spectrum of the polysaccharide (Table 1). It was characterised by the least sum of squared deviations of chemical shifts in the predicted and experimental spectra ($S = 0.6$ after normalisation to one sugar residue), while other theoretically possible structures with the given sugar composition had significantly higher S values (> 1.5).

→ 4)-α-D-Man *p*-(1 → 3)-β-D-Rha *p*^{II}

-(1 → 4)-β-D-Rha *p*^{I}-(1 → **1**

To confirm this structure, the ^{1}H NMR spectrum of the polysaccharide was completely assigned (Table 2) using COSY and H,H-relayed COSY experiments, and then the ^{13}C NMR spectrum was assigned using a ^{13}C, ^{1}H HETCOR experiment (Table 1). The $^{1}J_{C\text{-}1,H\text{-}1}$ coupling constant value determined from the gated-decoupling spectrum of the polysaccharide for both rhamnose residues was relatively small (159 Hz) and that for the mannose residue was relatively large (171 Hz). These data showed [9] that mannose is α-linked and both rhamnose residues are β-linked.

Downfield displacements of the signals for C-4 of Man and Rha^{I} and C-3 of Rha^{II} to δ 78.4, 83.6, and 81.9 in the ^{13}C NMR spectrum of the polysaccharide, as compared with their positions [7] in the spectra of the corresponding nonsubstituted monosaccharides at δ 68.2, 73.1, and 74.0, respectively, confirmed the substitution pattern.

Although the data of a 2D ROESY experiment could not be interpreted unambiguously owing to coincidence of some signals (e.g., those for H-3 of Rha^{I} and Rha^{II}), the spectrum displayed the expected cross-peaks which could be assigned to Man H-1, Rha^{II} H-3 at δ 5.03/3.61, Rha^{II} H-1, Rha^{I} H-4 at δ 4.64/3.43, and Rha^{I}, H-1, Man H-4 at 4.61/3.76.

Therefore, on the basis of the data obtained, it was concluded that the O-specific polysaccharide of *C. freundii* O3a,3b,1c has structure **1**. Remarkably, the

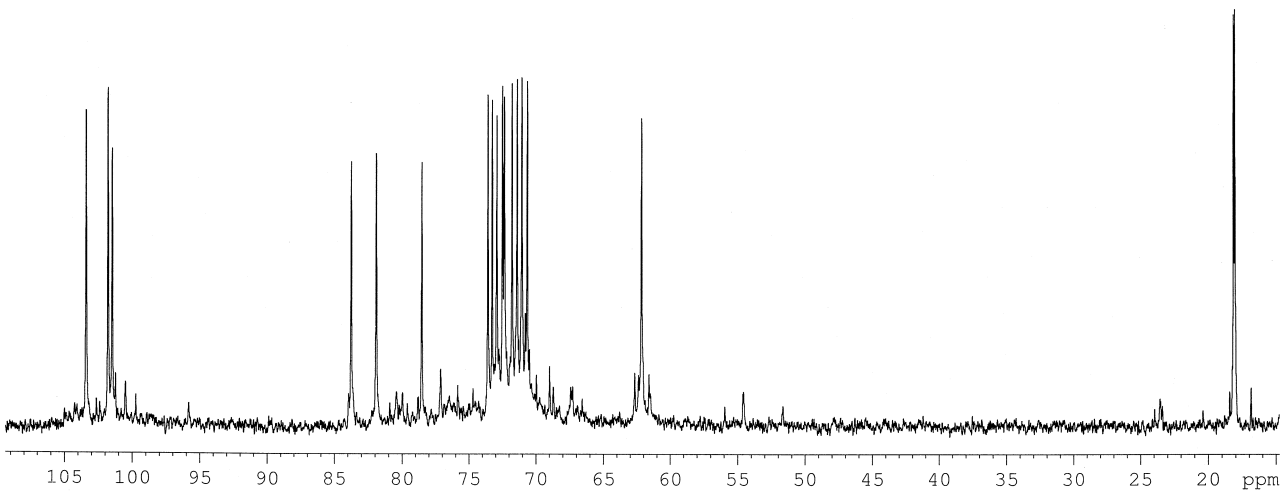

Fig. 1. 75 MHz ^{13}C NMR spectrum of the O-specific polysaccharide.

Table 2
300 MHz ^{1}H NMR data for the O-specific polysaccharide (δ in ppm)

	H-1	H-2	H-3	H-4	H-5	H-6
→ 4)-α-D-Man*p*-(1 →	5.03	4.04	3.92	3.76	3.83	3.73
→ 3)-β-D-Rha*p*^{II}-(1 →	4.64	4.16	3.61	3.40	3.39	1.25
→ 4)-β-D-Rha*p*^{I}-(1 →	4.61	4.02	3.62	3.43	3.47	1.25

polysaccharide includes D-rhamnose which is common to O-antigens of some phytopathogenic bacteria, such as *Pseudomonas syringae*, but uncommon to enterobacterial lipopolysaccharides [10].

1. Experimental

Bacterium, growth and isolation of LPS.—The bacterial culture of *Citrobacter* O3a,3b,1c (strain 35/57) was from the Czech National Collection of Type Cultures (Institute of Microbiology and Epidemiology, Prague). Growth of the bacterium in a dense agar medium [11], isolation of the lipopolysaccharide [5] and the O-specific polysaccharide [11] were performed as described.

Sugar analysis.—The polysaccharide was hydrolysed with 2 M CF_3CO_2H (121 °C, 1 h), released monosaccharides were converted into alditol acetates [12], and analysed by GLC on a Hewlett-Packard 5890A chromatograph equipped with a capillary column of Ultra 2. Absolute configurations of monosaccharides were determined by GLC of acetylated (*S*)-octyl glycosides by the published method [6].

NMR spectroscopy.—NMR spectra were run on a Bruker AM-300 spectrometer for solutions in deuterium oxide at 70 °C with acetone (δ_H 2.225, δ_C 31.45) as internal standard. Standard Bruker software was used in COSY and HETCOR experiments. A 2D ROESY experiment was carried out on a modified Bruker WM-250 spectrometer using the proposed pulse sequence [13] and a mixing time of 0.23 s; the HDO signal was suppressed by irradiation during 1 s.

Acknowledgements

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