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Note

Structure of the O-specific polysaccharide chain of *Citrobacter freundii* O28,1c lipopolysaccharide

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O-Specific polysaccharide chains of lipopolysaccharides of a number of serotypes of an enterobacterium, *Citrobacter freundii*, have been structurally elucidated [1–3]. We now report the structure of a new O-specific polysaccharide isolated from *C. freundii* O28,1c.

A neutral O-specific polysaccharide was obtained by mild acid degradation of the lipopolysaccharide isolated from bacterial cells by phenol–water extraction [4]. Sugar analysis using GLC of alditol acetates and acetylated (*S*)-2-octyl glycosides [5] revealed the presence in the polysaccharide of D-ribose and L-rhamnose in the ratio ca. 1:2.

The ^{13}C NMR spectrum of the polysaccharide was typical of a regular polymer (Fig. 1, Table 1). It contained signals for three anomeric carbons at δ 108.1, 103.1, and 102.0, for two methyl groups (C-6 of Rha) at δ 17.9 (2 C), one $-CH_2OH$ group (C-5 of Rib) at δ 63.0, and for 11 other sugar ring carbons in the region δ 70.3–84.1.

The ^1H NMR spectrum of the polysaccharide (Fig. 2, Table 2) contained signals for three anomeric protons at δ 5.36, 5.04, and 4.99 (all singlets), for two methyl groups (H-6 of Rha) at δ 1.31 and 1.29 (both doublets, $J_{5,6}$ 6 Hz), and for other sugar ring protons in the region δ 3.4–4.5.

These data suggested that the polysaccharide has a trisaccharide repeating unit containing two L-Rha and one D-Rib units.

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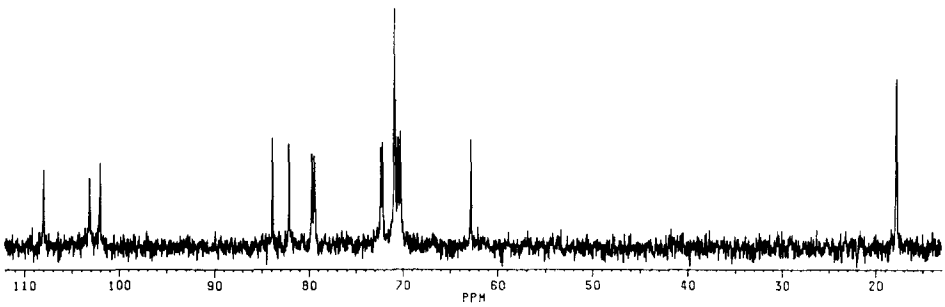

Fig. 1. ^{13}C NMR spectrum of *C. freundii* O28,1c O-specific polysaccharide.

The ^{1}H NMR spectrum of the polysaccharide was assigned using 2D shift-correlated spectroscopy (COSY) (Table 2) and, with the ^{1}H NMR spectrum assigned, the ^{13}C NMR spectrum of the polysaccharide was interpreted using heteronuclear $^{13}C-^{1}H$ COSY (Table 1). The chemical shifts, 108.1, 84.1, and 63.0 ppm, for C-1, C-4, and C-5 of Rib pointed to the β-furanosidic form for this sugar residue, while the chemical shift at δ 17.9 for C-6 of both Rha showed their pyranosidic form [6]. The relatively low-field position of the signals for C-3 of both Rha (I and II) at δ 79.9 and 79.4 and for C-2 of Rib at δ 82.2, compared with their positions in the spectra of the corresponding non-substituted monosaccharides [6], were caused by glycosylation and revealed the substitution pattern in the polysaccharide.

In 1D NOE experiments with sequential selective pre-irradiation of H-1 of all sugar residues, intraresidue NOEs were observed only on H-2 of the pre-irradiated sugar, thus indicating that both Rha moieties are α-linked (in the case of their β-configuration, NOEs on H-3 and H-5 would appear). In addition, the following interresidue NOEs on the transglycosidic protons were observed: H-1 Rha I/H-3 Rha II at δ 5.04/3.86, H-1 Rha II/H-2 Rib at δ 4.99/4.20, and H-1 Rib/H-3 Rha I at δ 5.36/3.90. These data were in agreement with the positions of substitution of the sugar residues determined by ^{13}C NMR spectroscopy and allowed determination of the following structure of the repeating unit of *C. freundii* O28,1c O-specific polysaccharide:

$$\rightarrow 3)\text{-}\alpha\text{-L-Rha}p\text{-}(1 \rightarrow 3)\text{-}\alpha\text{-L-Rha}p\text{-}(1 \rightarrow 2)\text{-}\beta\text{-D-Rib}f\text{-}(1 \rightarrow$$
$$\quad\quad\text{I}\quad\quad\quad\quad\quad\quad\text{II}$$

Like most O-specific polysaccharides of *C. freundii* studied previously [1–3], that of *C. freundii* O28,1c is neutral. Interestingly, the trisaccharide repeating unit of this

Table 1
^{13}C NMR chemical shifts for the O-specific polysaccharide (δ in ppm)

Sugar residue	C-1	C-2	C-3	C-4	C-5	C-6
→ 3)-α-L-Rha*p*-(1 → (I)	103.1	71.0	79.9	72.3	70.3	17.9
→ 3)-α-L-Rha*p*-(1 → (II)	102.0	71.0	79.4	72.5	70.6	17.9
→ 2)-β-D-Rib*f*-(1 →	108.1	82.2	71.0	84.1	63.0	

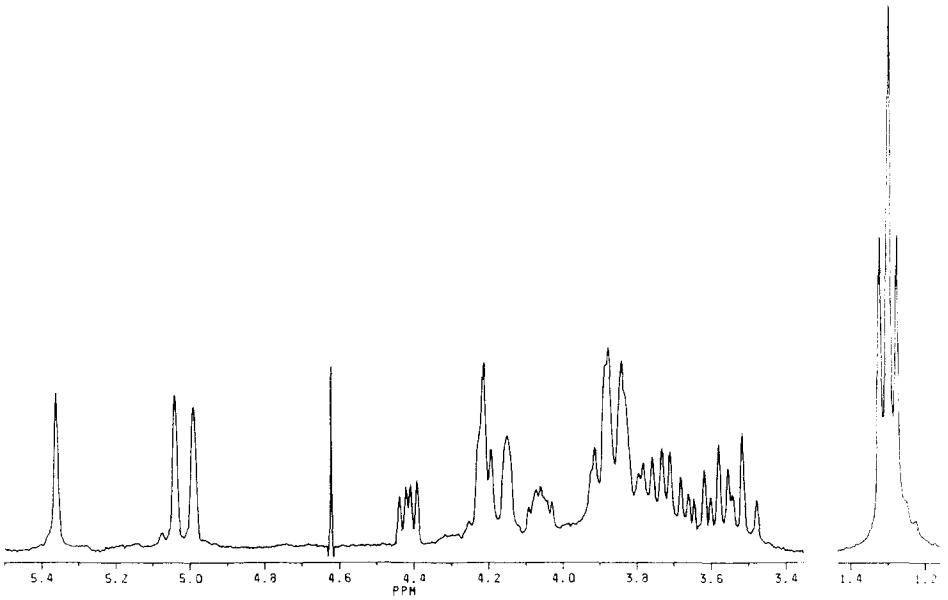

Fig. 2. ^{1}H NMR spectrum of *C. freundii* O28,1c O-specific polysaccharide.

polysaccharide is a part of the tetrasaccharide repeating unit of the O-specific polysaccharide of *Klebsiella* O7 [7].

1. Experimental

The ^{1}H and ^{13}C NMR spectra were obtained with a Bruker WM-250 instrument in D_2O at 40°C. Acetone was used as an internal standard (δ_H 2.23, δ_C 31.45). One-dimensional NOE, 2D COSY, and heteronuclear ^{13}C–^{1}H COSY (XHCORRD) experiments were performed using standard Bruker software. A mixing time of 1 s was used in 1D NOE experiments.

GPC was carried out on a column (40 cm × 2.5 cm) of Sephadex G-50 in pyridine acetate buffer (pH 4.5) and monitored with a Knauer differential refractometer. GLC

Table 2
^{1}H NMR chemical shifts for the O-specific polysaccharide (δ in ppm)

Sugar residue	H-1	H-2	H-3	H-4	H-5	H-6
→ 3)-α-L-Rha*p*-(1 → (I)	5.04	4.22	3.90	3.52	3.90	1.29 [a]
→ 3)-α-L-Rha*p*-(1 → (II)	4.99	4.15	3.86	3.58	3.79	1.31 [a]
→ 2)-β-D-Rib*f*-(1 →	5.36	4.20	4.42	4.07	3.86, 3.69	

[a] Assignments can be interchanged within column.

was performed using a Hewlett–Packard 5890 instrument equipped with a glass capillary column (25 m × 0.2 mm) coated with OV-1 stationary phase.

C. freundii O28,1c, strain 92/57 was obtained from the National Collection of Reference Strains (Institute of Hygiene, Prague). Growth of bacteria [8], isolation of lipopolysaccharide [4] and O-specific polysaccharide [8] were performed as described.

For sugar analysis, the polysaccharide (1 mg) was hydrolysed with 2 M CF_3COOH (120°C, 2 h), the hydrolysate was evaporated to dryness, sugars were conventionally reduced with $NaBH_4$ or converted to 2-octyl glycosides [5], acetylated, and analysed by GLC.

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