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The new sulfated O-specific polysaccharide from marine bacterium *Cobetia pacifica* KMM 3878, containing 3,4-O-[(*S*)-1-carboxyethylidene]-D-galactose and 2,3-O-disulfate-D-galactose

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ABSTRACT

The O-specific polysaccharide was isolated from the lipopolysaccharide of *Cobetia pacifica* KMM 3878 and studied by chemical methods along with ^{1}H and ^{13}C NMR spectroscopy, including, 1D TOCSY and 2D ^{1}H, ^{1}H COSY, ^{1}H, ^{13}C HSQC, ^{1}H, ^{1}H ROESY, ^{1}H, ^{13}C HMBC and ^{1}H, ^{13}C H2BC experiments. The following new structure of the sulfated O-polysaccharide from *C. pacifica* KMM 3878 containing 3,4-O-[(*S*)-1-carboxyethylidene]-D-galactose and 2,3-O-disulfate-D-galactose was established:

→4)-β-D-Gal2,3R-(1 → 6)-β-D-Gal3,4(S-Pyr)-(1 → 6)-β-D-Gal-(1→

Where R is $-SO_3H$.

1. Introduction

Gram-negative bacteria are an indispensable component of marine environments and represent a significant part of the ocean microbial community. The outer membrane of Gram-negative bacteria is the first and an immediate line of defense against harsh environment and antimicrobial molecules.

Lipopolysaccharides (LPSs), that compose a huge part of the outer membrane of Gram-negative bacteria, represent the contact between the bacterial cell and the surrounding environment and play an essential role in the adaptation of the organisms to peculiar environmental conditions. The chemical structure of the carbohydrate moiety of the marine Gram-negative bacteria LPSs is highly diverse and includes an ever-extending number of rare and unusual monosaccharides and non-carbohydrate substituents.[1] In this connection, the study of LPSs primary structure is important in order to understand the chemical modifications of the cell envelope to reinforce the external membrane and to ensure their survival.

Previously, we established the structure of the O-polysaccharide from the *Cobetia pacifica* KMM 3879^{T} and showed that the repeating unit of this polymer presented a branched trisaccharide containing rhamnose, 3-O-sulfate-D-glucose and 3-O-sulfate-D-galactose residues.[2] The main aim of this study was structural investigation of O-specific polysaccharide (OPS) of second strain of this species *C. pacifica*—*C. pacifica* KMM 3878, isolated from a sandy sediment sample collected at a depth of 1 m from the shore of the Sea of Japan.[3]

2. Result and discussion

Wet cells of *C. pacifica* KMM 3878 were treated with aqueous saline, to remove the capsular polysaccharide, which was not investigated further. The LPS was isolated from the treated cells by the phenol–water procedure[4] and degraded under alkaline conditions using aqueous ammonia treatment or under mild acidic conditions at pH 4.5. Comparison of the ^{13}C NMR spectra of both products showed that their polysaccharide parts had the identical repeating unit. Further studies were performed with the OPS obtained at alkaline conditions which gave better-resolved NMR spectra. GLC and GLC–mass spectrometry analysis of the acetylated alditols after hydrolysis of the OPS revealed only galactose residue

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(Gal) but analysis of the acetylated methyl glycosides after methanolysis revealed Gal and 3,4-O-[1-carboxyethylidene]-galactose residues in the ratio 2:1 (the full identification of this monosaccharide was completed by NMR spectroscopy, see below).

Mass-spectrum of acetylated methyl-ester of 3,4-O-[1-carboxyethylidene]-galactose after methanolysis and its fragment ions are given in Figure 1. Besides, OPS contained sulfate ester groups (22.9%), which were determined by turbidimetric method (see below).

The ^{1}H NMR spectrum of OPS (Table 1) contained, *inter alia*, six signals in anomeric region (three anomeric protons at δ_H 4.45-4.78 ppm and three ring proton at δ_H 4.51-4.61 ppm, see below) and one methyl group at δ 1.59 ppm (s).

The ^{13}C NMR spectrum of the OPS (Fig. 2, Table 1) contained, *inter alia*, three signals in anomeric region at δ_C 103.0–104.6 ppm, one non-substituted CH_2OH and two substituted OCH_2–C groups at δ_C 61.6 and 70.1, 71.2 ppm correspondingly (C-6 of Gal^{I}, Gal^{II}, and Gal^{III} residues, respectively, from data DEPT-135 spectrum). In the spectrum, there were three signals, from which one, at δ_C 24.6 ppm, belonged to a methyl group and the two others, at δC 109.1 and 178.8 ppm, to quaternary carbons (data DEPT-135 spectrum). These data demonstrated a trisaccharide repeating unit of OPS and suggested the presence of an acetal-linked pyruvic acid residue.[5] The absence from the ^{13}C NMR spectrum of any signals for non-anomeric sugar carbons in the region δ_C 82–88 ppm demonstrated the pyranose form of all sugar residues.[6]

The $^{1}J_{C1\text{-}H1}$ coupling constant values determined from the gated-decoupling spectrum of the OPS confirmed that the all sugar residues are in pyranoid form[8] and are β-linked (160.7–166.3 Hz).[7]

The ^{1}H and ^{13}C NMR spectra of the OPS were assigned using 1D TOCSY, 2D homonuclear ^{1}H, ^{1}H-COSY, ^{1}H, ^{1}H-ROESY, heteronuclear ^{1}H, ^{13}C-HSQC, ^{1}H, ^{13}C-HMBC, and ^{1}H, ^{13}C-H2BC experiments (Table 1).

The COSY and TOCSY spectra revealed proton spin-systems for three sugar residues having the *galacto* configuration by H-1/H-2 up to H-4 and H-6/H-5 correlations.

The ^{1}H, ^{13}C-HSQC experiment (Fig. 3.) revealed three anomeric signals, numerous ring signals, and one methyl group signal. The three sugar spin systems were identified as Gal^{I}, Gal^{II}, and Gal^{III} residues. Due to poor resolution of H-2 and H-3 signals, for assignment of C-2, C-3, and C-4 carbons of Gal^{I} residue ^{1}H, ^{13}C-H2BC experiment was used (Fig. 4, Table 1). The presence of H-1/C-2 correlation at δ_C 4.78/77.2 ppm suggested that signal of C-2 carbon of the Gal^{I} residue was at δ_C 77.2 ppm. The presence of H-3/C-2 correlation at δ_C 4.52/77.2 ppm indicated that signal of C-3 carbon was at δ_C 79.1 ppm. Correspondingly, signal of C-4 carbon of the Gal^{I} residue was at δ_C 74.8 ppm. The presence of pyruvic acid residue was confirmed by correlation of methyl group at δ_H 1.59 and the signals of quaternary carbons at δ_C 109.1 and 178.8 ppm founded from the ^{1}H, ^{13}C-HMBC spectra.

The position of substitution and the sequence of the sugar residues were established using ^{1}H, ^{1}H-ROESY, and ^{1}H, ^{13}C-HMBC (Fig. 5) experiments. The Gal^{I} residue was linked at C-6 of the Gal^{II} residue, as shown by inter-residual NOE connectivity H-1 of Gal^{I}/H-6 of Gal^{II} at δ_H/δ_H 4.78/3.99 ppm and by HMBC long range correlations H-1, C-1 of Gal^{I}/C-6, H-6 of Gal^{II} at δ_H/δ_C 4.78/70.1, δ_C/δ_H 103.0/3.99 ppm, respectively. The Gal^{II} residue was linked at C-6 of the Gal^{III} residue, as revealed by NOE connectivity H-1 of Gal^{II}/H-6 of Gal^{III} at δ_H/δ_H 4.45/3.86 ppm and by HMBC long range correlations H-1 of Gal^{II}/C-6 of Gal^{III} at δ_H/δ_C 4.45/71.2 ppm, respectively. The Gal^{III} residue, in turn, was linked to C-4 of the Gal^{I} residue, given the NOE connectivity H-1 of Gal^{III}/H-4 of Gal^{I} at δ_H/δ_H 4.74/4.61 ppm and the HMBC long range correlations H-1, C-1 of Gal^{III}/C-6, H-6 of Gal^{I} at δ_H/δ_C 4.74/74.8, δ_C/δ_H 104.6/4.61 ppm, respectively. These data defined the sequence of the monosaccharides and demonstrated that the Gal^{II} and Gal^{III} residues were 6-substituted, while the Gal^{I} residue was 4-substituted.

The ^{1}H and ^{13}C NMR chemical shifts for the pyruvic acid and Gal^{II} residues were in agreement with published data for 3,4-O-[1-carboxyethylidene]-β-D-galactopyranose. (α-Effects on C-3 and C-4 were +5.6 and +6.2 ppm, respectively, C-1 of pyruvic acid at δ_C 109.1 being typical of a 1,3-dioxolane ring).[5,8] The location of the pyruvic acid residue also was confirmed using HMBC experiment. It was established, that proton at C-3 of Gal^{II} residue correlated with quaternary carbon of pyruvic acid residue at δ_H/δ_C 4.22/109.1 ppm. These data suggested that pyruvic acid is acetal-linked to O-3 and O-4 of Gal^{II} residue.

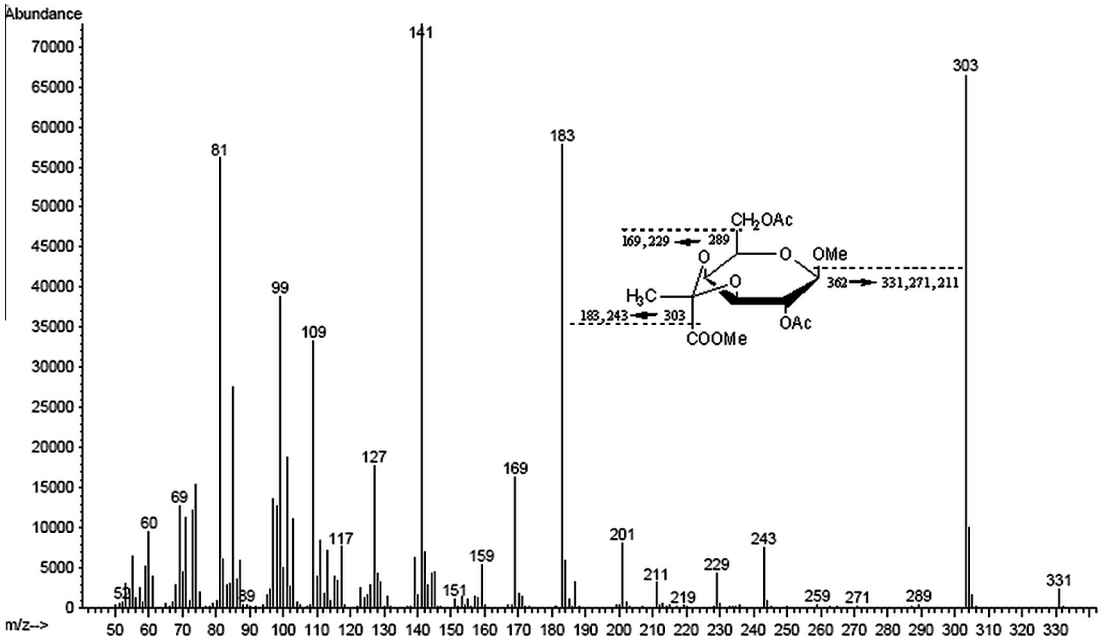

Figure 1. Mass-spectrum of acetylated methyl-ester of 3,4-O-[1-carboxyethylidene]-galactose after methanolysis.