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Note

Smith degradation of the O-antigenic polysaccharide of *Salmonella* Dakar: structural studies of the products

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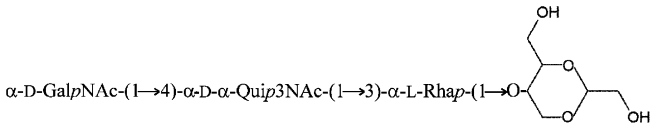
Abstract—Two different oligosaccharides were obtained from the Smith degradation of the O-polysaccharide isolated from the lipopolysaccharide of *Salmonella* Dakar. The structures of these oligosaccharides were investigated by chemical analysis, NMR spectroscopy and MALDI-TOF mass spectrometry. The following structures of these products were determined: α-D-Gal*p*NAc-(1→4)-α-D-Qui*p*3NAc-(1→3)-α-L-Rha*p*-(1→2)-threitol and



where Qui*p*3NAc is 3-acetamido-3,6-dideoxyglucose. The reaction products confirmed the structure of the repeating unit of the *Salmonella* Dakar O-polysaccharide reported previously [Kumirska, J.; Szafranek, J.; Czerwicka, M.; Paszkiewicz, M.; Dziadziuszko, H.; Kunikowska, D.; Stepnowski, P. *Carbohydr. Res.* **2007,** *342*, 2138–2143].

Keywords: Smith degradation; *Salmonella* Dakar; O-Polysaccharide; NMR; Mass spectrometry

This paper presents a method for the isolation and full structural investigation of two products obtained from the Smith degradation of the O-polysaccharide chain of the *Salmonella* Dakar lipopolysaccharide (*S.* Dakar OPS); the specific degradation technique most frequently used in structural investigations of polysaccharides.[1–3] The periodate oxidation of the OPS was performed according to the Pritchard and Rener's procedure[4] to substantiate its assigned structure.[5]

Sugar analysis of the periodate-oxidised and $NaBH_4$-reduced *S.* Dakar OPS (red.–ox. *S.* Dakar OPS) revealed the presence of ethane-1,2-diol, threitol, rhamnitol, 3-acetamido-3,6-dideoxyglucitol, galactosaminitol and glucitol in a molar ratio of 0.3:1.2:1.0:1.0:1.0:0.2. The substitution patterns of Rha at C-3, GalNAc at C-3 and C-4 and Qui3NAc at C-4 protected those sugars from periodate oxidation. A trace of Glc was probably derived from the glucosyl residue present in the core region.

The red.–ox. *S.* Dakar OPS was mildly hydrolysed. The reaction products were fractionated by GPC on a Bio-Gel P-2 column. The isolated oligosaccharide fraction was additionally separated by HPLC and two products were obtained; their MALDI mass spectra are shown in Figures 1 and 2. An additional sugar analysis of the minor product was also performed.

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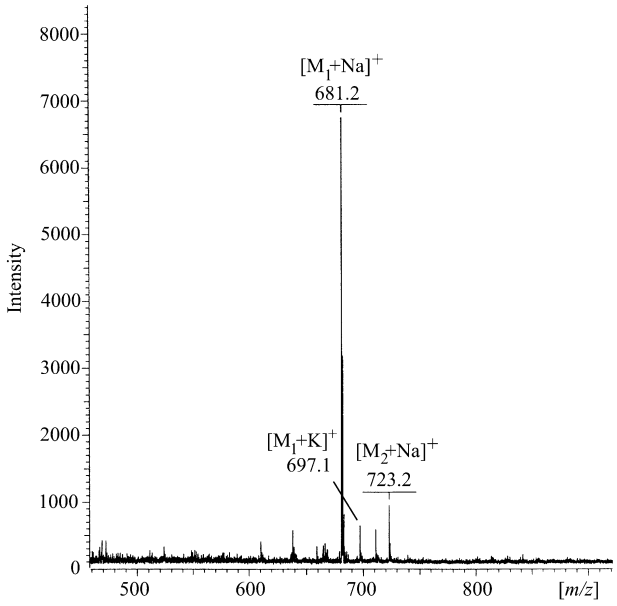


Figure 1. MALDI mass spectrum of the major product obtained from the Smith degradation of the *Salmonella* Dakar O-polysaccharide.

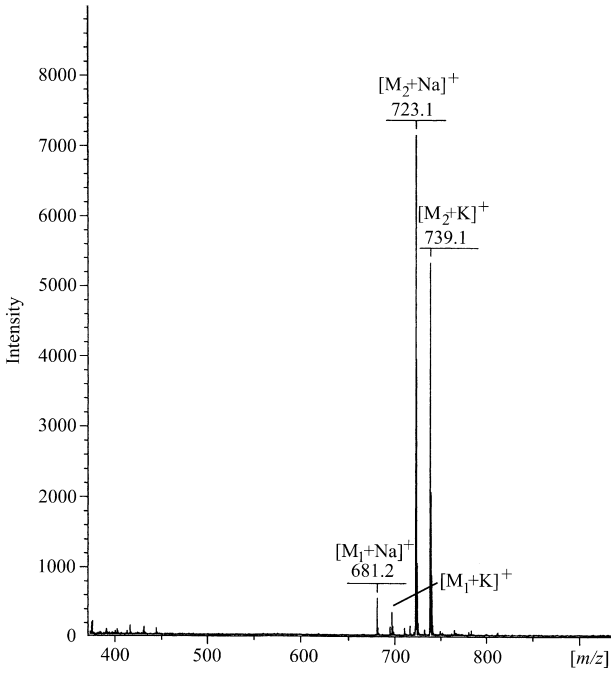


Figure 2. MALDI mass spectrum of the minor product obtained from the Smith degradation of the *Salmonella* Dakar O-polysaccharide.

The results of the sugar analysis of the red.–ox. *S.* Dakar OPS as well as the *m/z* values of 681.1 (M_1+Na) and 697.1 (M_1+K) given by the MALDI spectrum of the major product (Fig. 1) revealed the presence of a trisaccharide built from Rha, GalNAc and Qui3NAc and threitol (derived from the degraded Gal residue). The ^{13}C NMR spectrum of this product (Fig. 3) contained signals for three anomeric carbons, three HO*C*H_2–C groups, two *C*H_3–C groups, two nitrogen-bearing carbons, twelve non-anomeric oxygen-bearing sugar-ring carbons and resonances for two *N*-acetyl groups. The absence of signals at 84–88 ppm indicated that all the sugars were in the pyranose form.[6] The ^{1}H NMR spectrum (Fig. 4) contained three anomeric resonances at almost equal intensities, and signals for two *N*-acetyl and two methyl groups. The complete assignment of the chemical shift spin systems of the sugar residues and threitol presented in this product (Table 1) was achieved from the results of 2D NMR experiments, according to the published methodology.[7,8] The chemical shifts for C-4 of Qui3NAc (δ 76.31), C-3 of Rha (δ 76.32) and C-2 of threitol (δ 77.77), demonstrated the glycosylation pattern of these sugar residues.[6] The lack of the down field carbon signals for GalNAc in the ^{13}C NMR spectrum (Table 1) indicated that this sugar residue was present at the non-reducing end of this trisaccharide. The $^3J_{\text{H-1,H-2}}$ coupling constants (4.0 Hz for GalNAc and 3.6 Hz for Qui3NAc), the chemical shift of the H-1 protons (δ 5.29 for GalNAc, and δ 5.06 for Qui3NAc) and the chemical shift of the C-5 signal of Rha at δ 69.51,[9,10] confirmed α configurations for these monosaccharide residues. The monosaccharide sequence within the chain was established by NOESY and HMBC experiments (data not shown). In the NOESY spectrum, the following inter-residual NOE correlations were observed: GalNAc H-1, Qui3NAc H-4 at δ 5.29/3.54; Rha H-1, Threitol H-2 at δ 5.08/3.81; Qui3NAc H-1, Rha H-3 at δ 5.06/3.87, whereas in the HMBC spectrum between the following anomeric protons and linkage carbons: Rha H-1, Threitol C-2 at δ 5.08/77.77

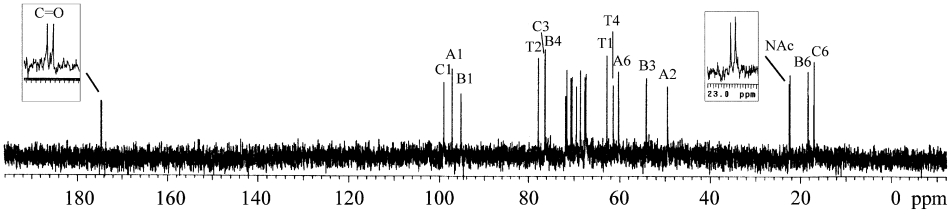


Figure 3. ^{13}C NMR spectrum (100 MHz) of the major product obtained from the Smith degradation of the *Salmonella* Dakar O-polysaccharide. The detailed description of the abbreviations used is presented in Table 1.

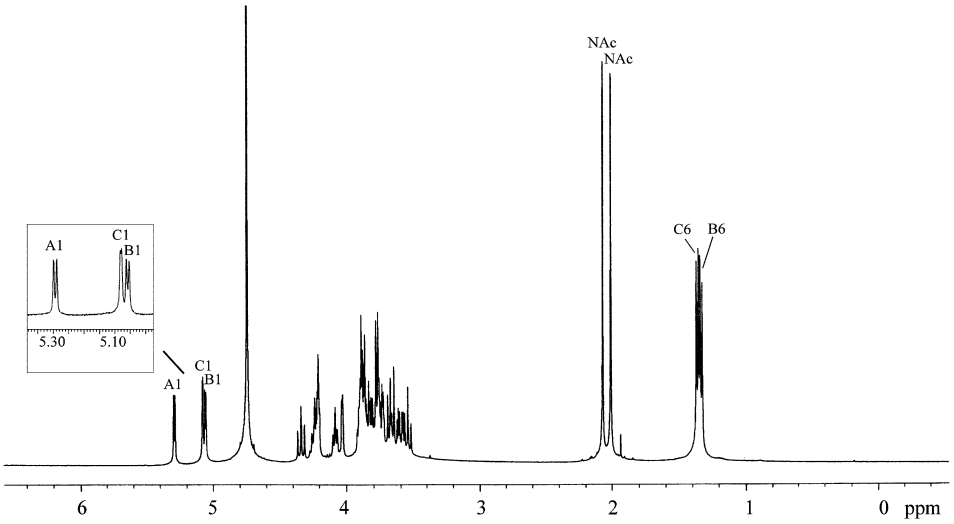


Figure 4. ^{1}H NMR spectrum (400 MHz) of the major product obtained from the Smith degradation of the *Salmonella* Dakar O-polysaccharide. The detailed description of the abbreviations used is presented in Table 1.

Table 1. ^{1}H and ^{13}C NMR chemical shifts (δ) for the major product obtained from the Smith degradation of the *Salmonella* Dakar O-polysaccharide

Sugar residue		H-1 C-1	H-2 C-2	H-3 C-3	H-4 C-4	H-5 C-5	H-6 C-6
α-D-Gal*p*NAc-(1→	**A**	5.29 97.17	4.21 49.56	3.86 67.55	4.03 68.66	3.89 71.53	3.85, 3.75 60.28
→4)-α-D-Qui*p*3NAc-(1→	**B**	5.06 95.18	3.60 70.66	4.34 54.13	3.54 76.31	4.23 67.38	1.33 18.24
→3)-α-L-Rha*p*-(1→	**C**	5.08 99.02	4.22 67.69	3.87 76.32	3.65 70.44	3.89 69.51	1.36 16.96
→2)-Threitol	**T**	3.73, 3.69 62.86	3.81 77.77	4.08 71.84	3.78 61.44		

There are additional chemical shifts for NAc in the ^{1}H NMR spectrum at δ 2.01 and 2. 07 for CH_3; also in the ^{13}C NMR spectrum at δ 22.40 and 22.57 for CH_3, and at δ 174.67 and 175.54 for C=O.

and Qui3NAc H-1, Rha C-3 at δ 5.06/76.32. Based on all the data, the major product obtained from the Smith degradation of the *S*. Dakar O-polysaccharide has the following structure:

α-D-Gal*p*NAc-(1→4)-α-D-Qui*p*3NAc-(1→3)-α-L-Rha*p*-(1→2)-threitol

Analogous structural studies were performed on the second product (23.4%) obtained from the Smith degradation of the *S*. Dakar O-polysaccharide. The MALDI spectrum of this compound (Fig. 2) showed two major ions: with *m*/*z* 723.1 (M_2+Na) and with *m*/*z* 739.1 (M_2+K), whereas the sugar analysis of this oligosaccharide revealed the presence of ethane-1,2-diol, threitol, rhamnitol, 3-acetamido-3,6-dideoxyglucitol and *N*-acetyl-galactosaminitol, in the ratios 0.3:1.0:1.1:0.9:1.0, respectively.

The ^{13}C NMR spectrum (Fig. 5) of the second product of the Smith degradation of *S*. Dakar OPS contained two additional signals: one in the anomeric region and one at δ 60.0–78.00 if compared to the ^{13}C NMR spectrum of the previous product. The absence of signals at 84–88 ppm (Fig. 5) again indicated six-membered rings in all the monosaccharides.[6] In the anomeric region of the ^{1}H NMR spectrum (Fig. 6), one additional resonance at δ 4.92 was also shown; the TOCSY spectrum (data not shown) indicated that it was correlated only with the resonance at δ 3.66. The 1D NMR data and 2D NMR experiments enabled the complete chemical shift spin systems of three monosaccharide residues—GalNAc, Qui3NAc and Rha (Table 2). The TOCSY

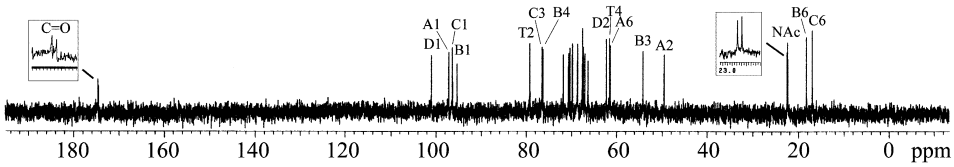


Figure 5. ^{13}C NMR spectrum (100 MHz) of the minor product obtained from the Smith degradation of the *Salmonella* Dakar O-polysaccharide. The detailed description of the abbreviations used is presented in Table 2.

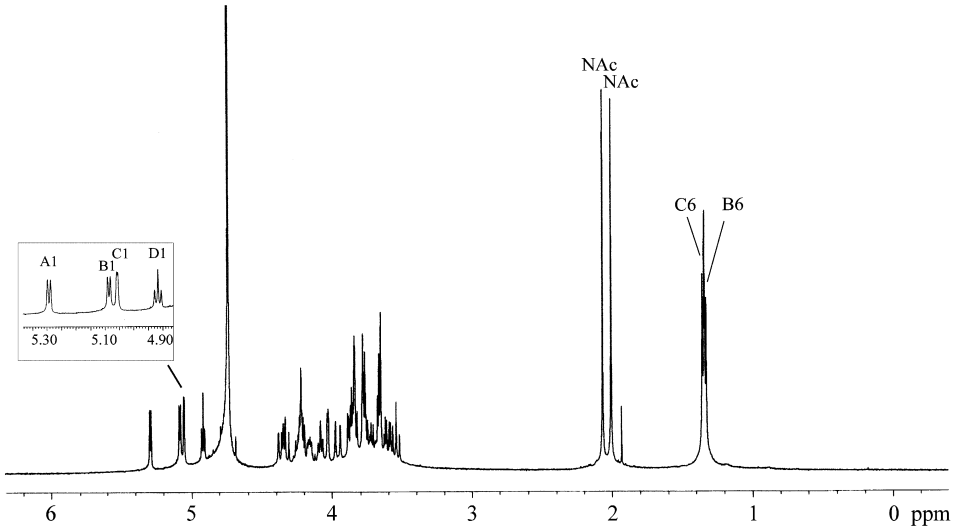
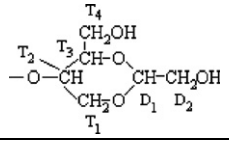


Figure 6. ^{1}H NMR spectrum (400 MHz) of the minor product obtained from the Smith degradation of the *Salmonella* Dakar O-polysaccharide. The detailed description of the abbreviations used is presented in Table 2.

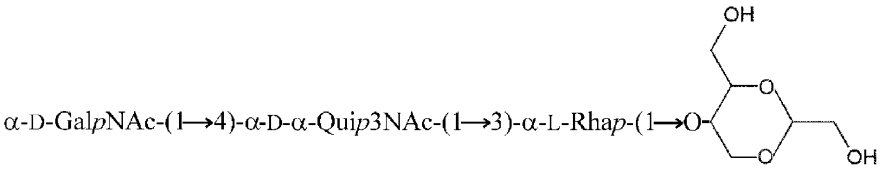
Table 2. ^{1}H and ^{13}C NMR chemical shifts (δ) for the minor product obtained from the Smith degradation of the *Salmonella* Dakar O-polysaccharide

Sugar residue		H-1 C-1	H-2 C-2	H-3 C-3	H-4 C-4	H-5 C-5	H-6 C-6
α-D-Gal*p*NAc-(1→	**A**	5.29 97.16	4.21 49.55	3.88 67.36	4.03 68.66	4.08 71.82	3.76 61.42
→4)-α-Qui*p*3NAc-(1→	**B**	5.09 95.28	3.60 70.63	4.34 54.12	3.55 76.28	4.24 67.54	1.33 18.24
→3)-α-L-Rha*p*-(1→	**C**	5.06 96.33	4.22 67.59	3.86 76.49	3.65 70.32	3.72 69.79	1.35 16.95
		3.96, 4.36 (T_{1a}), (T_{1b}) 66.36	4.16 (T_2) 79.14	3.84 (T_3) 67.03	3.79 (T_4) 61.62	4.92 (D_1) 100.95	3.66 (D_2) 62.32

There are additional chemical shifts for NAc in the ^{1}H NMR spectrum at δ 2.00 and 2.07 for CH_3; also in the ^{13}C NMR spectrum at δ 22.39 and 22.56 for CH_3, and at δ 174.66 and 175.53 for C=O.

spectrum contained also a signal at δ 3.84 that was correlated with the resonances at δ 4.36, 4.16 and 3.79. Moreover, in the TOCSY spectrum, there were correlations from the proton at δ 4.36 to the protons at δ 3.96 and 3.84. Identification of threitol and ethane-1,2-diol in sugar analysis and 2D NMR experiments clearly indicated that the minor product of the Smith degradation of *S*. Dakar OPS contained 1,3-*O*-(2′-hydroxyethylidene)threitol formed from the degraded Gal residue (Table 2). In the HMBC spectrum (data not shown), intra-residual correlations between H-1b of threitol (T_{1b}) and the carbons C-2 (T_2), C-3 (T_3) and C-1′ of the 2′-hydroxyethylidene group (D_1) at δ 4.36/79.14, 4.36/67.03 and 4.36/100.95, respectively were observed. In addition, the cross-peak H-1′ (D_1), C-2 (T_2) at δ 4.92/79.14 was also shown. The ^{13}C NMR data (Table 2) revealed down field signals at δ 76.28, 76.49 and 79.14 for C-4 of Qui3NAc, C-3 of Rha and C-2 (T_2) of

1,3-*O*-(2′-hydroxyethylidene)threitol, respectively, demonstrating the substitution pattern of these sugar residues and the cyclic acetal in this compound.[6] The lack of the down field carbon signals for GalNAc in the ^{13}C NMR spectrum (Table 2) indicated the terminal position of this sugar residue in the oligosaccharide chain. The α anomeric configuration of all residues were also established. The sequence of the sugar residues in this oligosaccharide was determined by NOESY and HMBC experiments. In addition, in the HMBC spectrum, the correlation between the H-1 of Rha and C-2 (T_2) of 1,3-*O*-(2′-hydroxyethylidene)threitol at δ 5.06/79.14 was also observed. Therefore, the minor product obtained from the Smith degradation of the *S.* Dakar O-polysaccharide has the following structure:



This cyclic acetal is probably formed during the mild acid hydrolysis of the red.–ox. *S.* Dakar OPS, wherein the hydrated hydroxyethanal moiety (from C-1 and C-2 of reduced and oxidised Gal residue) can appear and its non-hydrated form undergoes acid-catalysed transacetalation to give this product. The mechanism of this reaction was presented by Aspinall,[3] and, for example, the formation of the hydrated form of glyceraldehyde moiety from 2-substituted periodate-oxidised and $NaBH_4$-reduced Rha was observed by Perepelov et al.[11]

1. Experimental

The *S.* Dakar strain, growth conditions, isolation of the lipopolysaccharide and the separation of the O-polysaccharide chain from LPS were described in the previous paper.[5] The polysaccharide (24.1 mg) was oxidised with 40 mM $NaIO_4$ (3.6 mL) (Lancaster, Germany) in the dark at 4 °C for 120 h according to the mentioned procedure.[4] The reaction was terminated with ethylene glycol (0.5 mL), and the product was dialysed against distilled water (Spectra-Por 1000 Da, Spectrum Laboratories Inc., USA), then lyophilised. After reduction and neutralisation of the excess of $NaBH_4$ with aq 50% HOAc, the product was dialysed again and lyophilised (17.1 mg of the product was obtained). A portion of 0.5 mg was subjected to sugar analysis[5] and analysed by GLC and GLC–MS. The GLC analysis was performed on a GC 8000 TOP (CE Instruments) gas chromatograph equipped with a capillary column Sol–Gel (30 m, 0.25 mm I.D., 0.25 μm film thickness, SGE) using a linear temperature programme from 100 °C to 260 °C at 4 °C min^{-1}, then isothermally at 260 °C for 5 min. The GLC–MS analysis was carried out on a TRIO-2000 instrument (VG Biotech, UK) with an electron impact ionisation energy of 70 eV, coupled with a Hewlett–Packard 5890 gas chromatograph using the same capillary column and the same temperature programme. For sugar identifications, GLC analyses with standard co-injections and mass spectra were employed. The red.–ox. *S.* Dakar OPS (16.6 mg) was hydrolysed with aq 2% HOAc (100 °C, 2 h). The oligosaccharide fraction was isolated by GPC on a Bio-Gel P-2 (200–400 mesh, BioRad, Richmond, USA) column (100 × 0.9 cm) in pyridine–acetic acid buffer (pyridine–HOAc–water, 2:5:493, v/v/v) with flow rate 3.5 mL h^{-1}, and then rechromatographed by HPLC on a semi-preparative LiChrosorb—100 NH_2 column (300 mm × 8 mm, 5 μm, Knauer) using CH_3CN–H_2O (70:30, v/v) as eluent at a flow rate of 1 mL min^{-1}. The chromatograph was equipped with a differential refractometric detector (RIDK 102, Prague, Czech Republic). As a result, this fraction was separated into two products (23.4% and 52.1% of the oligosaccharide fraction, respectively); sugar analysis of the minor product was performed. Both the products were subjected to NMR and MALDI mass spectrometric studies according to the described conditions.[5] The mass spectra were carried out from *m/z* 200 to 1000 and were reported as the results of 60–180 laser shots. The NMR spectra for both samples were recorded with a Varian Mercury 400 MHz spectrometer in D_2O solutions at 30 °C. The mixing times of 110 ms and 300 ms were used in the TOCSY and NOESY experiments, respectively.

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