Structure of the Oligosaccharide Chain of the SR-Type Lipopolysaccharide of *Ralstonia solanacearum* Toudk-2

Evelina L. Zdorovenko,*,† Evgeny Vinogradov,‡ Kerstin Wydra,§ Buko Lindner,⊥ and Yuriy A. Knirel†

N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 119991 Moscow, Russia, Institute for Biological Sciences, National Research Council Canada, Ottawa, K1A 0R6, Canada, Institute of Plant Diseases and Plant Protection, University of Hannover, D-30167 Hannover, Germany, and Research Center Borstel, Center for Medicine and Biosciences, D-23845 Borstel, Germany

Received March 31, 2008; Revised Manuscript Received May 26, 2008

Aiming at improving classification and taxonomy of Gram-negative phytopathogenic bacteria, we studied the structure of the lipopolysaccharide of *Ralstonia solanacearum*. Mild acid hydrolysis of the lipopolysaccharide of strain Toudk-2 followed by gel chromatography resulted in an O-polysaccharide and two oligosaccharide fractions. The smallest-size oligosaccharide fraction was studied by sugar analysis, high-resolution electrospray ionization mass spectrometry, and, after fractionation by anion-exchange chromatography on HiTrap Q, by one- and two-dimensional ^{1}H and ^{13}C NMR spectroscopy. It was found that the isolated oligosaccharides consist of the lipopolysaccharide core with one O-polysaccharide repeat (O-unit) attached. The core exists in two major glycoforms differing from each other in a lateral octulosonic acid residue, which is either D-*glycero*-D-*talo*-oct-2-ulosonic acid or 3-deoxy-D-*manno*-oct-2-ulosonic acid. A peculiar feature of the core is the occurrence of 4-amino-4-deoxy-L-arabinose nonstoichiometrically linked to a heptose residue. The full structures of the core and the biological O-unit as well as the site of the attachment of the O-unit to the core were established.

Introduction

Ralstonia solanacearum is a devastating plant pathogen with a global distribution and an unusually wide host range. It also survives as a saprophyte in water or in the soil in the absence of host plants. The species *R. solanacearum* is heterogeneous with respect to biological and biochemical properties of individual strains. The systematics of this species is not yet clear; its representatives have long been assigned to the genus *Pseudomonas*. Thus, according to the ninth edition of *Bergey's Manual*,[1] the species *R. solanacearum* is assigned to the *Pseudomonas* RNA homology group II.[2] Yabuuchi et al.[2] investigated representatives of this group with the aim of revising their taxonomic position. Based on 16S rRNA sequence analysis, DNA−DNA hybridization, composition of cellular lipids and fatty acids, and phenotypic characteristics, it was suggested to transfer the representatives of the RNA homology group II from the genus *Pseudomonas* to a new genus *Burkholderia* with the type species *B. cepacia*. Later studies provided evidence for distinguishing the species *B. solanacearum* and *B. pickettii* from other species of the genus *Burkholderia*, and a new genus, *Ralstonia* (named in honor of the author who described the species *P. pickettii*), was established.[3]

The lipopolysaccharide (LPS) is the major surface glycoconjugate of *R. solanacearum*, which plays an important role in interaction of the bacterium with its host plant. The LPS is composed of lipid A, a core oligosaccharide, and an O-polysaccharide (O-antigen) built up of oligosaccharide repeating units (O-units). Biochemical, structural, and functional peculiarities of the LPS are considered to be an important chemotaxonomic criterion and may be useful for clarifying the intraspecific classification of *R. solanacearum* and its interrelations with other species. Structures of the O-polysaccharides of a number of smooth (S-type) strains of *R. solanacearum* have been determined.[4−10] Aiming at solving problems of recognition, taxonomy and classification of *R. solanacearum* strains, we established the structure of the LPS core region of strain Toudk-2 as reported in this paper.

* To whom correspondence should be addressed. E-mail: evelina@ioc.ac.ru.
† Zelinsky Institute.
‡ Institute for Bioogical Sciences.
§ University of Hannover.
⊥ Research Center Borstel.

Materials and Methods

Bacterial Strain, Growth, and Isolation of Lipopolysaccharide. *R. solanacearum* strain Toudk-2, race 1 biovar 3 was received from Thailand (Thaveechai, Kasetsart University, Bangkok). For the production of the preculture, a single bacterial colony grown for 48 h at 28 °C on NGA agar medium (ingredients per L: beef extract 3 g, Bacto peptone 5 g, D-glucose 2.5 g, agar 15 g) was transferred to 30 mL of nutrient broth (ingredients per L: yeast extract 1 g, Bacto peptone 10 g, casamino acid 1 g, glucose 10 g, proteose peptone 10 g). After 24 h of growth at 28 °C under stirring, 30 mL of the culture was transferred to 1000 mL of nutrient broth and grown to midlogarithmic phase for 30 h at 28 °C in 2 L Erlenmeyer flasks. The cells were harvested by centrifugation at 12000 *g* for 15 min. The pellets were resuspended by swirling in a solution containing 0.1% (w/v) NaCl and 10 mM EDTA, pH 7.0, and centrifuged at 10000 *g* for 20 min at 4 °C. The washing steps were repeated at least five times to remove the adsorbed exopolysaccharides. Bacterial pellets were killed by addition of sodium azide and lyophilized. LPS was isolated by phenol-water extraction[24] and purified by ultracentrifugation (100000 × *g*, 4 h, 2 times). The supernatant was dialyzed against distilled water and lyophilized.

Mild Acid Degradation of the Lipopolysaccharide. The LPS was dissolved in aqueous 1% HOAc and heated for 1.5 h at 100 °C. The precipitate was removed by centrifugation (13000 × *g*, 10 min), and one polysaccharide and two oligosaccharide fractions (OSI and OSII) were isolated from the supernatant by fractionation using gel-permeation chromatography on a column (40 × 2.6 cm) of Sephadex G-50

10.1021/bm800326u CCC: $40.75
Published on Web 07/10/2008

Chart 1. Structures of the O-Polysaccharide and Oligosaccharides 1−3 from OSII Isolated from the LPS of *R. solanacearum* Toudk-2[a]

O-polysaccharide

~15% β-Xyl
1
↓
4

→3)-α-Rha-(1→2)-α-Rha-(1→3)-α-Rha-(1→3)-β-GlcNAc-(1→

OSII

β-Ara4N **S**
1
↓

T **Z** **Y** **X** **K** **F** 7 **E** **C**
α-Rha-(1→2)-α-Rha-(1→3)-α-Rha-(1→3)-β-GlcNAc-(1→3)-α-Rha-(1→3)-α-Hep-(1→3)-α-Hep-(1→5)-Kdo **1**
2 4
↑ ↑
1 1
α-Rha **J** β-Glc **H**

T **Z** **Y** **X** **K** **F** **E** **C**
α-Rha-(1→2)-α-Rha-(1→3)-α-Rha-(1→3)-β-GlcNAc-(1→3)-α-Rha-(1→3)-α-Hep-(1→3)-α-Hep-(1→5)-Kdo **2**
2 4
↑ ↑
1 1
α-Rha **J** β-Glc **H**

β-Ara4N **S**
1
↓

T **Z** **Y** **X** **K** **F** 7 **E** **C**
α-Rha-(1→2)-α-Rha-(1→3)-α-Rha-(1→3)-β-GlcNAc-(1→3)-α-Rha-(1→3)-α-Hep-(1→3)-α-Hep-(1→5)-Kdo **3**
2 4 4
↑ ↑ ↑
1 1 2
α-Rha **J** β-Glc **H** α-Ko **D**

[a] All monosaccharides are in the pyranose form; Rha, Xyl, and Ara4N have the L-configuration, Glc and GlcNAc have the D-configuration.

(Amersham Biosciences, Sweden) in pyridinium acetate buffer (4 mL of pyridine and 10 mL of HOAc in 1 L of water) at a flow rate 30 mL·h^{-1}. Eluates were monitored with a differential refractometer (Knauer, Germany). The oligosaccharide fraction eluted from Sephadex G-50 last (OSII) was further fractionated by anion-exchange chromatography on a 5-mL column HiTrap Q (Amersham Biosciences, Sweden) using a linear gradient of 0→1 M NaCl over 1 h at a flow rate 3 mL·min^{-1} using pulsed amperometric detection (Dionex) for monotoring. Desalting was performed by gel filtration on a column (50 × 1.6 cm) of Sephadex G-15.

Chemical Analyses. For analysis of amino components, OSII was hydrolyzed with 80 μL of 4 M HCl at 100 °C for 16 h and applied as the phenylisothiourea derivatives to HPLC on a reversed-phase Pico-Tag column (150 × 3.9 mm) using buffers for Pico-Tag amino acid analysis of protein hydrolysates (Waters, Germany) at 42 °C and a flow rate 1 mL·min^{-1} for 10 min; monitoring was performed with a dual λ absorbance detector (Waters, Germany) at 254 nm. For sugar analysis, OSII was hydrolyzed with 2 M CF_3CO_2H (120 °C, 2 h), the monosaccharides were analyzed by GLC as the alditol acetates on a Hewlett-Packard 5880 instrument (U.S.A.) equipped with an Ultra 2 capillary column and a temperature gradient of 180 °C (1 min) to 290 at 10 °C·min^{-1}. Methanolysis of OSII was performed with 2 M HCl in MeOH at 85 °C for 2 h, the products were conventionally acetylaed and analyzed by GLC/MS on a Hewlett_Packard HP 5989A instrument equipped with an HP-5 ms and a temperature gradient of 150 °C (3 min) to 320 at 5 °C·min^{-1}.

Electrospray Ionization Mass Spectrometry. Negative ion ion-cyclotron resonance Fourier transform ESI MS was performed using a mass analyzer (ApexII, Bruker Daltonics, U.S.A.) equipped with a 7 T actively shielded magnet and an Apollo electrospray ion source. Mass spectra were acquired using standard experimental sequences as provided by the manufacturer. Mass scale was calibrated externally with Re-LPS of known structure. Samples were dissolved in a 50:50:0.001 (v/v/v) mixture of 2-propanol, water, and triethylamine at a concentration of ~10 ng·μL^{-1} and sprayed at a flow rate of 2 μL·min^{-1}. Capillary entrance and exit voltage was set to 3.8 kV and −100 V, respectively; the drying gas temperature was 150 °C. The spectra showing several charge states for each component were charge deconvoluted using Bruker xmass 6.0.0 software, and mass numbers given refer to monoisotopic molecular masses.

NMR Spectroscopy. NMR spectra were obtained on a Varian UNITY/Inova 500 spectrometer (U.S.A.) in 99.96% ^2H_2O at 35 °C using internal acetone (δ_H 2.225, δ_C 31.45) as reference. Prior to the measurements, the samples were lyophilized twice from ^2H_2O. Mixing times of 120 and 300 ms were used in TOCSY and ROESY experiments, respectively.

Results

The LPS was isolated by phenol-water extraction and purified by ultracentrifugation. Mild acid degradation of the LPS followed by gel-permeation chromatography on Sephadex G-50 afforded the O-polysaccharide and two oligosaccharides fractions (OSI and OSII).

A comparison of the ^{13}C NMR spectrum with published data[6] showed that the isolated O-polysaccharide has essentially the same structure as that of *R. solanacearum* ICMP 7960. It has the main chain with a tetrasaccharide repeat (O-unit) containing one 2-acetamido-2-deoxy-D-glucose (GlcNAc) and three L-rhamnose residues, and ~15% repeat bears an L-xylose residue (Chart 1). Judging from electrospray ionization (ESI) mass spectra, the isolated oligosaccharides correspond to the LPS core with mostly one (OSII) or several (OSI) O-units attached. OSI was not studied further.

Analysis of sugar composition of OSII by GLC of the alditol acetates and amino acid analyzer after full acid hydrolysis revealed rhamnose (Rha), glucose (Glc), L-*glycero*-D-*manno*-heptose (Hep), 2-amino-2-deoxyglucose (GlcN), and 4-amino-4-deoxyarabinose (Ara4N) in the ratios ~9.5:3:4:6:2. The same monosaccharides and, in addition, 3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo) and a disaccharide composed of Kdo and D-*glycero*-D-*talo*-oct-2-ulosonic acid (Ko) were identified by

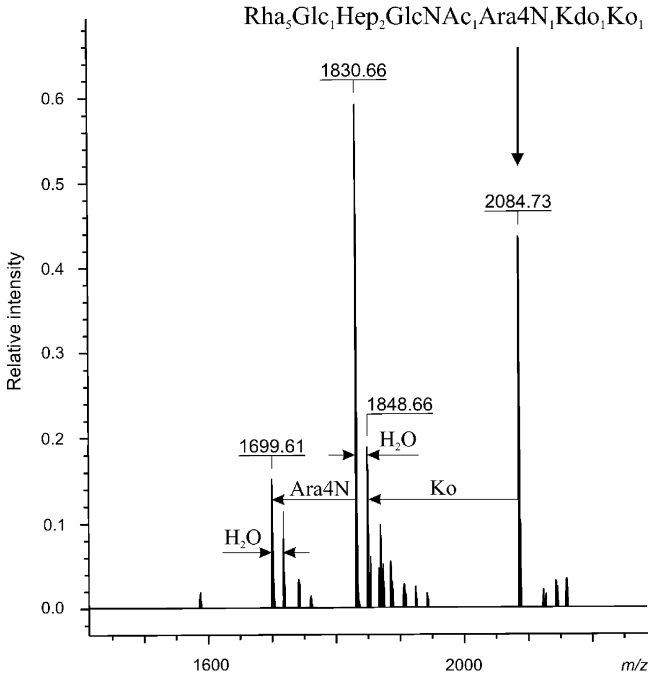

Figure 1. Charge-deconvoluted negative ion ESI mass spectrum of OSII isolated from the LPS of *R. solanacearum* Toudk-2.

GLC-MS of the acetylated methyl glycosides derived after methanolysis with 2 M HCl/MeOH.

Negative ion ion-cyclotron resonance Fourier transform ESI mass spectrum of OSII (Figure 1) showed major multiple-charged pseudomolecular ions derived from compounds with molecular masses 1699.61, 1830.66, 1848.66, and 2084.73 Da. Taking into account the sugar analysis data of the oligosaccharides, the highest-mass peak at 2084.73 u could be assigned to the compound having the composition $Rha_5Glc_1Hep_2HexNAc_1Ara4N_1Kdo_1Ko_1$ (calculated molecular mass 2084.73 Da). The other compounds have no Ko (Δm 236) or no Ara4N (Δm 131 u) or differ by dehydratation of Kdo at the reducing end (Δm 18 u). Therefore, the individual oligosaccharides in OSII differ in the presence or absence of Ko and Ara4N and the occurrence of Kdo in either normal or anhydrous form.

OSII was fractionated by anion-exchange chromatography on HiTrap Q column to give three fractions designated in the order of elution as **1** (front), **2**, and **3** (retained). The ^{13}C NMR spectrum of each oligosaccharide showed signals for anomeric carbons at δ 96.8−103.9, methylene group (C3) of Kdo at δ 35.0−35.2, methyl group (C6) of multiple Rha residues at δ 17.6−18.5, a nitrogen-bearing carbon (C2) of GlcN at δ 56.9, other sugar carbons at δ 61.7−82.6, and an *N*-acetyl group at δ 23.5 (CH_3). The ^{13}C NMR spectra of **1** and **3** contained a signal at δ 53.3 for an additional nitrogen-bearing carbon (C4) of Ara4N. The ^1H NMR spectrum of each oligosaccharide (parts of the spectrum for **3** are shown in Figure 2) contained signals for anomeric protons at δ 4.55−5.28, methylene group (H3) of Kdo at δ 1.92 or 2.06, methyl group (H6) of Rha residues at δ 1.21−1.31, other sugar protons at δ 3.28−4.35, and an *N*-acetyl group at δ 2.03. The observed single H3 signal for Kdo does not look as either axial or equatorial and, most likely, belongs to an anhydro form of Kdo.

The ^1H and ^{13}C NMR signals of the oligosaccharides **1**−**3** were assigned by two-dimensional NMR spectroscopy, including COSY, TOCSY, ROESY (parts of the spectra for **3** are shown in Figure 2), and ^1H,^{13}C HMQC experiments, and spin systems for 5 Rha, 1 Glc, 2 Hep, 1 GlcNAc, and 1 Kdo were identified in all oligosaccharides (Table 1). The spectra of the oligosaccharides **1** and **3** also contained spin systems for 1 Ara4N and those of the oligosaccharide **3**, an additional spin system for 1 Ko. Therefore, the NMR data of the isolated oligosaccharides are in full agreement with the MS data of their mixture discussed above (Figure 1). For further studies the constituent monosaccharides were designated by letters as shown in Table 1 and Chart 1.

Typically, large coupling constants $^3J_{1,2}$ 7−7.5 Hz showed that Glc, GlcNAc, and Ara4N are β-linked. The β-configuration of Glc and GlcNAc was confirmed by the presence of intraresidue H1/H3 and H1/H5 cross-peaks for these monosaccharides in the ROESY spectrum (Figure 2). The α-configuration of all Rha and Hep residues followed from the ^1H and ^{13}C NMR chemical shifts typical of the corresponding α-pyranosides[11,12] and from the presence in the ROESY spectrum of H1/H2 cross-peaks with no H1/H3 and H1/H5 cross-peaks; likewise, the α-configuration of Ko in **3** was inferred by comparison with published chemical shift data.[13]

The substitution pattern of the sugar residues in the oligosaccharides **1**−**3** were inferred by downfield displacements of the ^{13}C NMR signals for the linkage carbons, which are caused by a spatial interaction of the protons at the anomeric and linkage carbons of the neighboring linked sugar residues and are called α-effects of glycosylation.[11] Thus, downfield shifts of the signals for C2 of Rha **Z**, c3 of Rha **K** and **Y**, and C3 of GlcNAc **X** to δ 78.5−82.6 (Table 1), that is, by 7−10 ppm compared to their positions in the corresponding nonsubstituted monosaccharides[11] revealed the glycosylation pattern of these monosaccharides. In the spectra of **1** and **3**, a similar glycosylation shift to δ 72.0−72.9 of the C7 signal of Hep **E**, as compared with its position at δ 64.5 in the spectrum of **2**, showed its glycosylation by Ara4N **S** at position 7. A comparison of the ^{13}C NMR chemical shift δ 73.2 for C4 of Kdo **C** in **3** with that in **1** and **2** (δ 66.9−67.1) indicated substitution of Kdo at position 4 by Ko. Furthermore, a low-field position of the C5 signal of Kdo **C** at δ 69.8 in **3** compared with δ 64.95 in a α-Ko-(2→4)-Kdo disaccharide[13] demonstrated glycosylation of Kdo **C** also at position 5. This is also the case of **1** and **2** in which, as expected, C5 of Kdo **C** resonates even at a lower field (δ 76.2−76.3) in the absence of the Ko substituent at O4. Lower downfield displacements by 3−7 ppm of the signals for C3 and C4 of Hep **E** and C3 of Hep **F** (compare data of Table 1 with published data[12]) were indicative of glycosylation of the heptose residues at the vicinal positions (that no downfield shift was observed for the signal for C2 of Hep **F** was accounted for by a strong β-effect of glycosylation at position 3, which caused an upfield shift of the C2 signal[11]).

Sequence analysis of **3** was performed using ROESY (Figure 2). Correlations of Hep **E** H1 with Kdo **c** H5 and H7 (Table 2) are characteristic for an α1→5-linkage.[14] Anomeric protons of the other constituent monosaccharides exhibited cross-peaks with protons at the linkage carbons of neighboring monosaccharide residues, which were assigned taking into account the ^{13}C NMR chemical shift data (see above) as follows: Rha **T** H1/Rha **Z** H2, Rha **Z** H1/Rha **Y** H3, Rha **Y** H1/GlcNAc **X** H3, GlcNAc **X** H1/Rha **K** H3, Rha **K** H1/Hep **F** H3, Rha **J** H1/Hep **F** H2, Hep **F** H1/Hep **E** H3, Glc **H** H1/ Hep **E** H4, and Ara4N **S** H1/ Hep **E** H7 (Table 2).

Rha **T** H1/Rha **Z** H1 and Rha **J** H1/Hep **F** H1 correlations further confirmed the 1→2-linkage between these two monosaccharide pairs. These data were sufficient to infer the full structure of the oligosaccharide **3** shown in Chart 1. Similar analysis using ROESY of oligosaccharides **1** and **2** showed that they have the

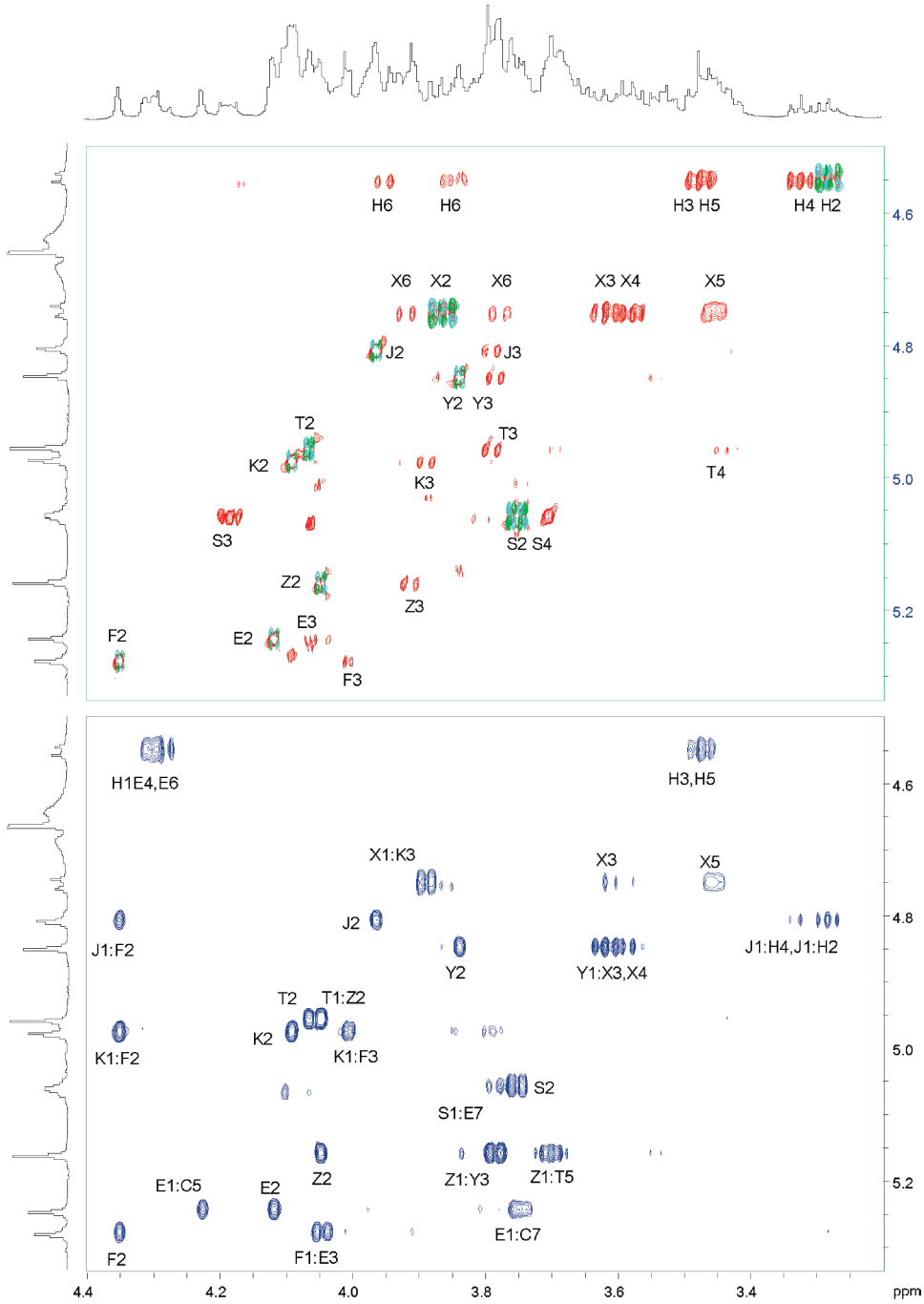

Figure 2. Parts of two-dimensional TOCSY (red), COSY (green) (both top), and ROESY (bottom) spectra of the oligosaccharide **3** from OSII isolated from the LPS of *R. solanacearum* Toudk-2.

same structures as **3** but lack Ko or both Ko and Ara4N, respectively (Chart 1).

Discussion

Mild acid hydrolysis of the LPS of *R. solanacearum* Toudk-2 released the O-polysaccharide and higher oligosaccharides. The former belongs to a large group of *R. solanacearum* O-polysaccharides[6] synthesized by polymerization of the same preassembled lipid-linked α-Rha-(1→2)-α-Rha-(1→3)-α-Rha-(1→3)-GlcNAc tetrasaccharide O-unit called biological O-unit.[15] The resultant O-polysaccharides may differ from each other in the linkage between the O-units, which is α1→2, α1→3, or β1→3,[6] depending on the specificity of O-antigen polymerase that catalyzes the polymerization.[15] Some of the O-polysaccharides remain linear and some others are nonstoichiometrically decorated by lateral α-L-rhamnosyl or, as the O-polysaccharide studied in this work, β-L-xylosyl groups.[6] A comparison of the isolated O-polysaccharide and oligosaccharide structures suggests that the distal α-Rha-(1→2)-α-Rha-(1→3)-α-Rha-(1→3)-β-GlcNAc tetrasaccharide fragment of the smallest-size oligosaccharides represents a single O-unit attached to the core. Hence, these oligosaccharides originated from a short-chain LPS consisting of core-lipid A with only one O-unit attached (so-called SR-type LPS). Elucidation of their full structures defines unambiguously the biological O-unit of *R. solanacearum* Toudk-2 with GlcNAc as the first monosaccharide, whose transfer to a lipid carrier initiates biosynthesis of the O-antigen.[15] It revealed also the site of the attachment of the O-unit to the

Table 1. ^{1}H and ^{13}C NMR Chemical Shifts of the Oligosaccharides **1**−**3** from OSII Isolated from the LPS of *R. solanacearum* Toudk-2 (δ, ppm)

compound, unit	H1 C1	H2 C2	H3 C3	H4 C4	H5 C5	H6 C6	H7 C7	H8 C8
1 →5)-Kdo (**C**)			1.92	4.12	4.11	3.88	3.70	3.67;3.87
			35.2	66.9	76.3	72.4	70.4	64.5
2 →5)-Kdo (**C**)			1.92	4.13	4.13	3.88	3.70	3.66;3.82
			35.2	67.1	76.2	72.5	70.5	64.6
3 →4,5)-Kdo (**C**)			2.06	4.12	4.23	3.81	3.70	3.58;3.77
			35.0	73.2	69.8	73.1	70.4	64.5
3 α-Ko-(2→ (**D**)			3.97	3.91	4.08	3.65	4.07	3.68;3.68
			73.6	67.8	69.6	73.5	71.4	64.3
1 β-Ara4N-(1→ (**S**)	5.03	3.78	4.19	3.68	3.78;4.08			
	100.1	69.4	67.2	53.0	59.7			
3 β-Ara4N-(1→ (**S**)	5.06	3.75	4.18	3.70	3.81;4.07			
	100.6	69.3	66.9	53.3	59.3			
1 →3,4,7)-α-Hep-(1→ (**E**)	5.09	4.15	4.18	4.30	4.15	4.31	3.72;3.90	
	102.3	71.7	74.6	74.7	73.2	68.3	72.0	
2 →3,4)-α-Hep-(1→ (**E**)	5.08	4.14	4.18	4.29	4.15	4.10	3.73;3.73	
	102.4	71.9	74.9	74.8	72.8	69.5	64.5	
3 →3,4,7)-α-Hep(1→ (**E**)	5.24	4.12	4.04	4.29	4.10	4.31	3.78;4.11	
	100.0	71.8	75.4	74.6	73.2	68.8	72.9	
1−**3** →2,3)-α-Hep-(1→ (**F**)	5.28	4.35	4.01	4.01	3.68	4.10	3.60;3.68	
	100.1	71.2	74.1	65.5	73.2	69.7	64.3	
1−**3** β-Glc-(1→ (**H**)	4.55	3.28	3.47	3.32	3.47	3.85;3.95		
	103.9	74.9	76.8	71.8	78.2	63.1		
1−**3** α-Rha-(1→ (**J**)	4.81	3.96	3.79	3.43	3.79	1.31		
	99.3	71.6	71.3	73.3	70.2	18.5		
1−**3** →3)-α-Rha-(1→ (**K**)	4.97	4.09	3.89	3.53	3.94	1.27		
	96.8	71.4	81.3	72.1	70.0	17.8		
1−**3** →3)-β-GlcNAc-(1→ (**X**)	4.75	3.87	3.62	3.58	3.46	3.78;3.92		
	103.1	56.9	82.6	69.3	76.8	61.7		
1−**3** →3)-α-Rha-(1→ (**Y**)	4.87	3.84	3.78	3.53	3.79	1.23		
	102.5	71.8	78.5	72.8	70.2	17.6		
1−**3** →2)-α-Rha-(1→ (**Z**)	5.16	4.05	3.91	3.48	4.02	1.31		
	102.1	79.2	71.2	73.3	70.2	17.9		
1−**3** α-Rha-(1→ (**T**)	4.96	4.07	3.79	3.43	3.70	1.25		
	103.5	71.4	71.3	73.3	70.4	17.8		x

Table 2. Interresidue Correleations Observed in the ROESY Spectrum of the Oligosaccharides **3**[a]

unit	^{1}H (anomeric)	unit	^{1}H NOE to
E	5.24	**C**	H5 4.23 (s), H7 3.70 (m)
S	5.06	**E**	H7 3.78 (m), 4.11 (w)
F	5.28	**E**	H3 4.04 (s)
H	4.55	**E**	H4 4.29 (s)
J	4.81	**F**	H1 5.28 (s), H2 4.35 (m)
K	4.97	**F**	H2 4.35 (s), H3 4.01 (s)
X	4.75	**K**	H3 3.89 (s)
Y	4.87	**X**	H3 3.62 (s), H4 3.58 (m)
Z	5.16	**Y**	H3 3.78 (s)
T	4.96	**Z**	H1 5.16 (w), H2 4.05 (s)

[a] s = strong, m = medium, and w = weak.

core. Remarkably, no xylose is present in the core-linked O-unit, which finding is in agreement with a nonstoichiometric (∼25%) content of xylose in the O-polysaccharide. Most likely, as lateral glucose residues in many O-antigens,[15] in *R. solanacearum* Toudk-2, and other *R. solanacearum* strains with Xyl-containing O-antigens,[6] the xylosyl groups are attached after polymerization. Another peculiar feature is that no noncapped core oligosaccharide was isolated and, hence, the population of the LPS species from the strain studied is devoid of R-type LPS.

In *R. solanacearum* Toudk-2, the LPS core itself is restricted to an octasaccharide consisting of 2 Rha, 2 Hep, 1 Glc, 1 Ara4N, 1 Ko, and 1 Kdo (or 2 Kdo, see below). A minority of the oligosaccharides (compound **2**) lacks Ara4N. Two major core glycoforms occur, one containing and the other lacking a terminal 2→4-linked α-Ko residue in the inner region (compounds **1** and **3**). Most likely, the latter was derived from a glycoform containing a terminal Kdo residue in place of the Ko residue. The glycosidic linkage of Kdo is known to be acid-labile and the putative terminal Kdo residue could be cleaved upon mild acid hydrolysis of the LPS to give the oligosaccharide **1**, whereas the glycosidic linkage of Ko is stable under the hydrolysis conditions used and, as a result, the oligosaccharide **3** was obtained. A similar alternation of Kdo and Ko in the LPS core has been reported in medically important bacteria *Yersinia pestis* and *Yersinia pseudotuberculosis*, incorporation of Ko being temperature-dependent and observed preferentially at lower cultivation temperatures (20−28 °C).[16,17] Remarkably, Ara4N in the core of *R. solanacearum* is linked to a heptose residue to yield a β-Ara4N-(1→7)-Hep disaccharide, which, to our knowledge, is reported for the first time in bacterial LPS. In the LPS core of a taxonomically related bacterium *Burkholderia cepacia*, a β-Ara*p*4N-(1→8)-α-Ko-(2→4)-Kdo trisaccharide was identified.[13]

In some other respects, the LPS core structure of *R. solanacearum* resembles that of *B. cepacia*[18] and *Burkholderia pyrrocinia*,[19] both bacteria sharing an α-Rha-(1→2)-α-Hep-(1→3)-[β-Glc-(1→4)]-α-Hep-(1→5)-[α-Ko-(2→4)]-Kdo hexasaccharide fragment. This structure similarity is in agreement with a close phylogenetic relationship of these bacteria belonging to the same family *Burkholderiaceae*. On the other hand, the core composition and structure of *R. solanacearum* differs from those of representatives of "true" pseudomonads belonging to the RNA homology group I, including *P. syringae*, *P. aeruginosa*, and *P. fluorescens*. Particularly, whereas the core of the *Pseudomonas* LPS is highly phosphorylated,[20–22] in all representatives of *Ralstonia* and *Burkholderia* studied so far (refs 18 and 23 and this work) the core is free of phosphate. These data demonstrate the expediency of the recent transfer of the

genus *Ralstonia* from the family *Pseudomadaceae* to the family *Burkholderiaceae*.

Acknowledgment. Authors thank H. Moll and A.N. Kondakova for help with GLC-MS and ESI MS, respectively, and A.S. Shashkov for measuring NMR spectra of the O-polysaccharide.

References and Notes

(1) Palleroni,. N J. In *Bergy's Manual of Systematic Bacteriology*; Krieg, N. R., Holt, J. G., Eds.; Williams & Wilkins: Baltimore, 1984; Vol. 1, pp 141−198.
(2) Yabuuchi, E.; Kosako, H.; Oyaizu, H.; Yano, I.; Hotta, H.; Hashimoto, Y.; Ezaki, T.; Arakawa, M. *Microbiol. Immunol.* **1992**, *36*, 1251–1275.
(3) Yabuuchi, E.; Kosako, H.; Yano, I.; Hotta, H.; Nishiuchi, Y. *Microbiol. Immunol.* **1995**, *39*, 807–904.
(4) Akiyama, Y.; Eda, S.; Kato, K.; Tanaka, H. *Carbohydr. Res.* **1984**, *133*, 289–296.
(5) Kocharova, N. A.; Knirel, Y. A.; Shashkov, A. S.; Kochetkov, N. K.; Varbanets, L. D. *Carbohydr. Res.* **1992**, *228*, 315–320.
(6) Kocharova, N. A.; Knirel, Y. A.; Shashkov, A. S.; Nifant'ev, N. E.; Kochetkov, N. K.; Varbanets, L. D.; Moskalenko, N. V.; Brovarskaya, O. S.; Muras, V. A.; Young, J. M. *Carbohydr. Res.* **1993**, *250*, 275–287.
(7) Bhattacharyya, T.; Basu, S. *Carbohydr. Res.* **1993**, *250*, 335–337.
(8) Shashkov, A. S.; Kocharova, N. A.; Knirel, Y. A.; Varbanets, L. D.; Moskalenko, N. V.; Zdokhlii, A. V. *Bioorg. Khim.* **1993**, *19*, 1089–1094.
(9) Kocharova, N. A.; Shashkov, A. S.; Knirel, Y. A.; Varbanets, L. D.; Moskalenko, N. V. *Carbohydr. Res.* **1994**, *259*, 153–157.
(10) Varbanets, L. D.; Kocharova, N. A.; Knirel, Y. A.; Moskalenko, N. V. *Biochemistry (Moscow)* **1996**, *61*, 580–585.
(11) Lipkind, G. M.; Shashkov, A. S.; Knirel, Y. A.; Vinogradov, E. V.; Kochetkov, N. K. *Carbohydr. Res.* **1988**, *175*, 59–75.
(12) Zamyatina, A.; Gronow, S.; Puchberger, M.; Graziani, A.; Hofinger, A.; Kosma, P. *Carbohydr. Res.* **2003**, *338*, 2571–2589.
(13) Isshiki, Y.; Kawahara, K.; Zähringer, U. *Carbohydr. Res.* **1998**, *313*, 21–27.
(14) Knirel, Y. A.; Grosskurth, H.; Helbig, J. H.; Zähringer, U. *Carbohydr. Res.* **1995**, *279*, 215–226.
(15) Raetz, C. R. H.; Whitfield, C. *Annu. Rev. Biochem.* **2002**, *71*, 635–700.
(16) Knirel, Y. A.; Lindner, B.; Vinogradov, E. V.; Kocharova, N. A.; Senchenkova, S. N.; Shaikhutdinova, R. Z.; Dentovskaya, S. V.; Fursova, N. K.; Bakhteeva, I. V.; Titareva, G. M.; Balakhonov, S. V.; Holst, O.; Gremyakova, T. A.; Pier, G. B.; Anisimov, A. P. *Biochemistry* **2005**, *44*, 1731–1743.
(17) Knirel, Y. A. Kondakova, A. N. Bystrova, O. V. Lindner, B. Shaikhutdinova, R. Z. Dentovskaya, S. V. Anisimov, A. P. *Adv. Sci. Lett.* **2008**, in press.
(18) Isshiki, Y.; Zähringer, U.; Kawahara, K. *Carbohydr. Res.* **2003**, *338*, 2659–2666.
(19) Silipo, A.; Molinaro, A.; Comegna, D.; Sturiale, L.; Cescutti, P.; Garozzo, D.; Lanzetta, R.; Parrilli, M. *Eur. J. Org. Chem.* **2006**, 4874–4883.
(20) Zdorovenko, E. L.; Vinogradov, E. V.; Zdorovenko, G. M.; Lindner, B.; Bystrova, O. V.; Shashkov, A. S.; Rudolph, K.; Zähringer, U.; Knirel, Y. A. *Eur. J. Biochem.* **2004**, *271*, 4968–4977.
(21) Knirel, Y. A.; Bystrova, O. V.; Kocharova, N. A.; Zähringer, U.; Pier, G. B. *J. Endotoxin Res.* **2006**, *12*, 324–336.
(22) Knirel, Y. A.; Helbig, J. H.; Zähringer, U. *Carbohydr. Res.* **1996**, *283*, 129–139.
(23) Molinaro, A.; De Castro, C.; Lanzetta, R.; Evidente, A.; Parrilli, M.; Holst, O. *J. Biol. Chem.* **2002**, *277*, 10058–10063.
(24) Westphal, O.; Jann, K. *Methods Carbohydr. Chem.* **1965**, *5*, 83–91.

BM800326U