Structure of the O-polysaccharide of *Erwinia carotovora* ssp. *atroseptica* GSPB 9205 containing a new higher branched monosaccharide*

S. N. Senchenkova,[a] A. S. Shashkov,[a] Yu. A. Knirel,[a] M. Ahmed,[b] A. Mavridis,[b] and K. Rudolph[b]*

[a]N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 47 Leninsky prosp., 119991 Moscow, Russian Federation. Fax: +7 (095) 137 6148. E-mail: knirel@ioc.ac.ru
*[b]Institute of Plant Pathology and Plant Protection, Georg August University, 6 Grisebachstr., D-37077 Göttingen, Germany.** Fax: (49) 551 39 8177. E-mail: amavrid@gwgd.de*

An O-polysaccharide was prepared by mild acid hydrolysis of a lipopolysaccharide of *Erwinia carotovora* ssp. *atroseptica* GSPB 9205 and was found to contain a new higher branched monosaccharide, *viz.*, 3,6,8-trideoxy-4-*C*-(*R*-1-hydroxyethyl)-D-*gulo*-octose, which we called erwiniose. The structure of the pentasaccharide repeating unit of the O-polysaccharide was established by monosaccharide analysis, including the determination of absolute configurations, methylation analysis, *O*-deacetylation, Smith degradation, and one- and two-dimensional NMR spectroscopy. The configuration of erwiniose was determined based on the coupling constants of vicinal protons combined with the nuclear Overhauser effect data and the results of periodate oxidation of the polysaccharide followed by reduction of the resultant 4-keto sugar (the C(1)—C(4)—C(1′)—C(2′) fragment) to give 3,6-dideoxy-D-*ribo*-hexose (paratose) and oxidation of 3-hydroxybutyraldehyde (the C(5)—C(8) fragment) to give *R*-3-hydroxybutyric acid.

Key words: lipopolysaccharide, polysaccharide structure determination, higher branched monosaccharide, *Erwinia carotovora*.

Lipopolysaccharide (LPS) forms the outer layer of the outer membrane of the Gram-negative bacteria cell walls. The fine structure of the O-polysaccharide chain of the LPS is responsible for intraspecies diversity of strains and specificity of interactions of bacterial cells with other biological systems. Classification schemes of many Gram-negative bacteria necessary for epidemiologic monitoring are based on O-polysaccharide structures.

Lipopolysaccharides of phytopathogenic bacteria are involved in plant pathogenesis, for example, in soft rot caused by *Erwinia carotovora* strains. The structure of LPS of bacteria belonging to the *Erwinia* genus was poorly studied.[1—5] The structures of O-polysaccharides were established only for two strains, *viz.*, *E. carotovora* ssp. *atroseptica* GSPB 436 [1] and *Erwinia amylovara* T.[4] The classification scheme of bacteria *Erwinia* is lacking. With the aim of classifying strains of phytopathogenic bacteria, we perform a series of structural studies of their O-polysaccharides. In the present study, we established the structure of the O-polysaccharide of *E. carotovora* ssp. *atroseptica* GSPB 9205. We found and identified a new higher branched monosaccharide in this O-polysaccharide and called it erwiniose.

Results and Discussion

Mild acid degradation of LPS, which was isolated from *E. carotovora* ssp. *atroseptica* GSPB 9205 cells by aqueous-phenol extraction, afforded an O-polysaccharide (**1**). Monosaccharide analysis by GLC of alditol acetates obtained upon total acid hydrolysis of polysaccharide **1** revealed the presence of galactose, mannose, and rhamnose in a ratio of 2 : 1 : 1. The GLC analysis of acetylated glycosides with an optically active alcohol, *viz.*, *S*-octan-2-ol, revealed D configuration for galactose and mannose and L configuration for rhamnose. Methylation analysis of the polysaccharide revealed 3-substituted galactose residues, a 4-substituted rhamnose residue, and a 2,3-disubstituted mannose residue. The presence of the latter monosaccharide is indicative of a branched structure of the polysaccharide. However, the terminal monosaccharide of the side chain was not detected.

* Dedicated to Academician N. K. Kochetkov on the occasion of his 90th birthday.

** Instutut für Pflanzenpathologie und Pflanzenschutz, Georg-August-Universität, Grisebachstr., 6, D-37077 Göttingen, Bundesrepublik Deutschland.

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 5, pp. 1239—1244, May, 2005.

1066-5285/05/5405-1276

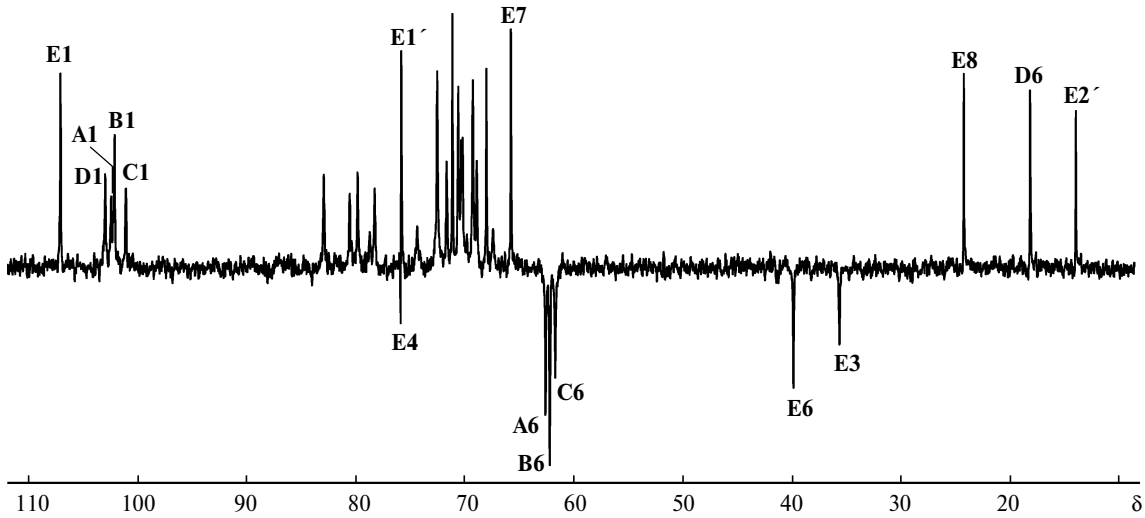


Fig. 1. ^{13}C NMR spectrum of the *O*-deacetylated polysaccharide recorded with the use of the attached proton test (APT) technique. The signals for the carbon atoms bearing an odd number of protons are directed upward, and the signals for the carbon atoms bearing an even number of protons are directed downward. The numbers refer to the carbon atoms in the monosaccharide residues.

The ^{13}C NMR spectrum of polysaccharide **1** suggests its regular structure consisting of pentasaccharide repeating units. The signal at δ 21.7, which disappears upon mild alkaline treatment of the polysaccharide, is indicative of the presence of an *O*-acetyl group. The ^{13}C NMR spectrum recorded using the attached proton test (APT) technique of *O*-deacetylated polysaccharide **2** (Fig. 1) has, in addition to signals of two galactose residues (**A** and **B**), one mannose residue (**C**), and one rhamnose residue (**D**), a number of other signals. Further investigation demonstrated that the latter signals belong to a higher monosaccharide, which we called erwiniose (**E**). These signals correspond to two methyl and two methylene groups at δ 13.9, 24.2, 35.6, and 39.9 (Table 1) and to one tertiary carbon atom bound to oxygen (δ 75.9), which indicates that erwiniose has a branched carbon skeleton.

O-Deacetylated polysaccharide **2** was studied by two-dimensional NMR spectroscopy, including ^{1}H,^{1}H-homonuclear COSY, TOCSY, and ROESY correlation experiments and ^{1}H,^{13}C-heteronuclear HSQC and HMBC experiments. As a result, we made the assignment of the signals for the protons of spin systems of two α-galactopyranose residues (**A** and **B**), an α-mannopyranose residue (**C**), and an α-rhamnopyranose residue (**D**) and three spin systems (H(1)—H(3), H(5)—H(8), and H(1′)—H(2′)) of erwiniose (Table 2). From the NMR data for erwiniose, it follows that its skeleton is branched at the C(4) atom, and the deoxy units are at positions 3 and 6 (CH_2 groups) and at positions 8 and 2′ (CH_3 groups)

Table 1. ^{13}C NMR spectroscopic data for *O*-deacetylated polysaccharide **2** and oligosaccharide **3**

Unit	δ					
	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)
	O-Deacetylated polysaccharide **2**					
→3)-α-D-Gal*p*-(1→ (**A**)	102.5	70.2	78.3	70.3	72.6	62.3
→3)-α-D-Gal*p*-(1→ (**B**)	102.2	68.9	79.9	70.6	72.6	62.6
→2,3)-α-D-Man*p*-(1→ (**C**)	101.1	80.6	78.8	67.4	74.4	61.7
→4)-α-L-Rha*p*-(1→ (**D**)	103.1	71.7	69.3	83.0	69.3	18.1
β-Erw*p*-(1→ (**E**)*	107.2	68.0	35.6	75.9	71.1	39.9
	Oligosaccharide **3**					
α-D-Gal*p*-(1→ (**A**)	102.2	70.0	70.6	70.6	72.6	62.5
→3)-α-D-Gal*p*-(1→ (**B**)	102.0	67.6	80.0	70.4	72.6	62.0
→2,3)-α-D-Man*p*-(1→ (**C**)	99.8	80.2	78.7	67.8	74.2	62.0
→3)-1-Deoxyerythritol (**D′**)	62.6	83.6	69.0	18.5	—	—
β-Par*p*-(1→ (**E′**)	107.0	69.5	39.1	70.9	77.0	18.0

* The following signals were also observed (δ): 65.8 (C(7)); 24.2 (C(8)); 75.8 (C(1′)); 13.9 (C(2′)).

Table 2. ^{1}H NMR spectroscopic data for *O*-deacetylated polysaccharide **2**

Unit	δ (^3J/Hz)						
	H(1)	H(2)	H(3) (H_{eq}(3))	H(4) (H_{ax}(3))	H(5)	H(6) (H(6a))	H(6b)
→3)-α-D-Gal*p*-(1→ (**A**)	5.25 ($J_{1,2}$ = 4)	3.93 ($J_{2,3}$ = 10)	3.95 ($J_{3,4}$ = 3)	4.06 ($J_{4,5}$ < 2)	4.11	3.85	3.85
→3)-α-D-Gal*p*-(1→ (**B**)	5.27 ($J_{1,2}$ = 4)	4.02 ($J_{2,3}$ = 10)	3.98 ($J_{3,4}$ = 3)	4.23 ($J_{4,5}$ < 2)	4.09	3.75	3.75
→2,3)-α-D-Man*p*-(1→ (**C**)	5.31 ($J_{1,2}$ < 2)	4.19 ($J_{2,3}$ = 3)	4.05 ($J_{3,4}$ = 10)	4.04 ($J_{4,5}$ = 10)	3.99	3.73	3.73
→4)-α-L-Rha*p*-(1→ (**D**)	5.06 ($J_{1,2}$ < 2)	4.06 ($J_{2,3}$ = 3.5)	3.94 ($J_{3,4}$ = 9.5)	3.53 ($J_{4,5}$ = 9.5)	3.90 ($J_{5,6}$ = 6)	1.30	—
β-Erw*p*-(1→ (**E**)*	4.58 ($J_{1,2}$ = 7.7)	3.75 ($J_{2,3_{eq}}$ = 5)	2.01 ($J_{2,3_{ax}}$ = 9)	1.70 ($J_{3_{eq},3_{ax}}$ = 10.4)	3.72 ($J_{5,6a}$ = 11.2)	1.53 ($J_{5,6b}$ < 2)	1.72 ($J_{6a,6b}$ = 13.8)

* The following signals were also observed (δ, *J*/Hz): 3.98 (H(7), $J_{6a,7}$ < 2); 1.24 (H(8), $J_{6b,7}$ = 10); 4.02 (H(1′), $J_{7,8}$ = 6.2); 1.18 (H(2′), $J_{1',2'}$ = 6.3).

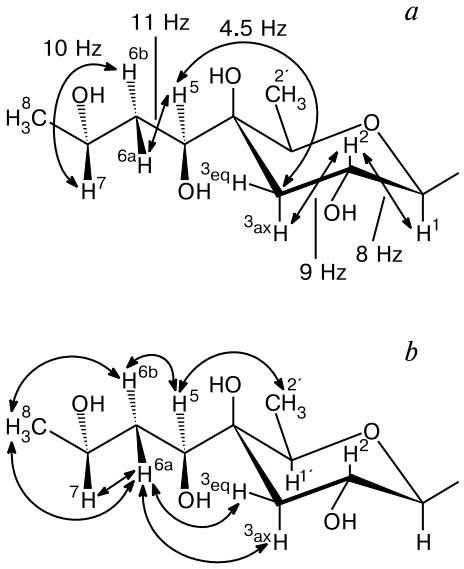


Fig. 2. Configuration and the preferable conformation of the erwiniose residue in the polysaccharide established by NMR spectroscopy. The mutual arrangement of the atoms based on the coupling constants (*a*) and the closely-spaced protons revealed by ROESY spectroscopy (*b*) are shown.

(Fig. 2). Analysis of the chemical shifts in the ^{13}C NMR spectrum, which were assigned based on the results of the HSQC experiment (see Table 1), confirmed these conclusions.

The glycosylation positions and the sequence of monosaccharide residues in the repeating unit of the polysaccharide were determined from the correlations between the anomeric protons and protons at the linkage carbons revealed in the ROESY experiment (Table 3). These data were confirmed by the HMBC spectrum, which demonstrated that there are correlations between the anomeric protons and the linkage carbons or between the anomeric carbon atoms and the linkage protons, respectively (data not presented). The conclusions about the structure of polysaccharide **2** (Scheme 1) are consistent with the results of methylation (see above) and show that the erwiniose residue is the terminal monosaccharide of the side chain.

A comparison of the chemical shifts of the signals in the ^{1}H NMR spectra of the original and *O*-deacetylated polysaccharides (**1** and **2**, respectively) revealed a substantial difference in the position of the signal for H(2) of the galactose residue **B** (δ 5.12 and 4.02, respectively). The low-field shift of this signal in the spectrum of the original polysaccharide is caused by the deshielding effect of the *O*-acetyl group, which is, therefore, located at position 2 of the unit **B**.

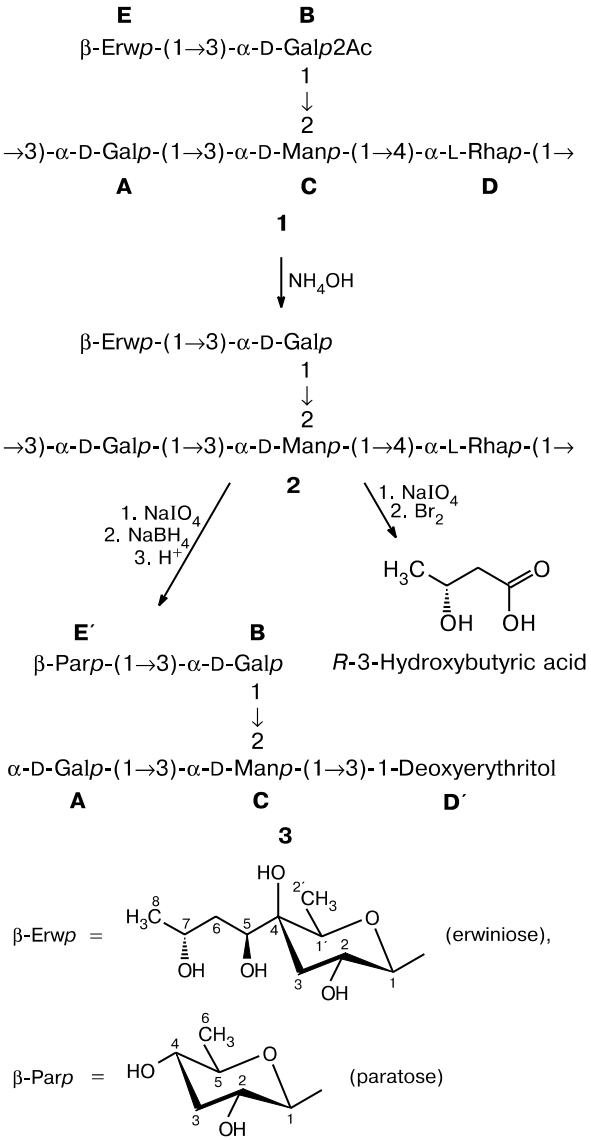
The NMR spectroscopic data allowed us also to determine the relative configuration of erwiniose. The relatively large coupling constants ($^3J_{H(1),H(2)}$ = 7.7 Hz and $^3J_{H(2),H_{ax}(3)}$ = 9 Hz) are indicative of the axial orientation of the H(1) and H(2) protons of the pyranose ring. The coupling constants $^3J_{H(5),H(6a)}$ = 11.2 Hz and $^3J_{H(6b),H(7)}$ = 10 Hz provide evidence that the preferable conformation is characterized by the *trans* arrangement of the H(5) and H(6a) protons and of the H(6b) and H(7) protons in the side chain (see Fig. 2, *a*). The coupling constant $^3J_{C(3),H(5)}$ = 4.5 Hz is also rather high, which is evidence for the *trans* orientation of the C(3) and H(5) atoms (see Fig. 2, *a*). The correlations between the closely arranged protons (see Fig. 2, *b*), which were revealed by analysis of the ROESY spectrum (Fig. 3), in particular, the H_{eq}(3)/H(6a), H_{ax}(3)/H(6a), H(5)/H(2′), H(5)/H(6b), H(6a)/H(7), H(6a)/H(8), and H(6b)/H(8) correlations, are consistent with these data and provide evidence for the configuration of erwiniose (see Fig. 2).

The structure and configuration of erwiniose were confirmed by chemical methods. *O*-Deacetylated polysaccharide **2** was subjected to periodate oxidation and the resultant 3-hydroxybutyraldehyde was oxidized with bro-

Table 3. Through-space correlations between the closely-spaced protons revealed in the ROESY experiment

Anomeric proton of the unit	Proton at the linkage carbon							
	H(3) (**A**)	H(2) (**C**)	H(3) (**C**)	H(4) (**D**)	H(3) (**B**)	H(2) (**D′**)	H(3) (**D′**)	H(4) (**D′**)
				O-Deacetylated polysaccharide **2**				
→3)-α-D-Gal*p*-(1→ (**A**)	–	–	+	–	–	–	–	–
→3)-α-D-Gal*p*-(1→ (**B**)	–	+	–	–	–	–	–	–
→2,3)-α-D-Man*p*-(1→ (**C**)	–	–	–	+	–	–	–	–
→4)-α-L-Rha*p*-(1→ (**D**)	+	–	–	–	–	–	–	–
β-Erw*p*-(1→ (**E**)	–	–	–	–	+	–	–	–
				Oligosaccharide **3**				
→3)-α-D-Gal*p*-(1→ (**A**)	–	+	+	–	–	–	–	–
α-D-Gal*p*-(1→ (**B**)	–	+	–	–	–	–	–	–
→2,3)-α-D-Man*p*-(1→ (**C**)	–	–	–	–	–	+	+	+
β-Par*p*-(1→ (**E′**)	–	–	–	–	+	–	–	–

Scheme 1



mine to give *R*-3-hydroxybutyric acid (see Scheme 1), which was identified as *O*-trifluoroacetylated *S*-2-octyl ester by GLC. Consequently, the C(7) atom in erwiniose has the D configuration.

The polysaccharide product obtained upon periodate oxidation was reduced with sodium borohydride and subjected to mild acid hydrolysis (Smith degradation, see Scheme 1). The resulting oligosaccharide **3** was studied by NMR spectroscopy, as was described above for the polysaccharide (Tables 1, 3, and 4). As expected, the galactose and mannose residues remained unoxidized, the rhamnose residue was transformed into 1-deoxyerythritol, and erwiniose was transformed into 3,6-dideoxyhexose. The relatively high coupling constants ($^3J_{H(2),H_{ax}(3)} \approx {}^3J_{H_{ax}(3),H(4)} = 11.5$ Hz and $^3J_{H(4),H(5)} = 9.3$ Hz) are indicative of the axial orientation of the H(2), H(4), and H(5) protons, which corresponds to the *ribo* configuration of the monosaccharide. Analysis of the glycosylation effects in the ^{13}C NMR spectrum of oligosaccharide **3**, in particular, the absence of the effect on the C(4) atom of the galactose residue **B** due to its glycosylation at position 3, demonstrated that the monosaccharides in the disaccharide fragment, *viz.*, 3,6-dideoxy-β-*ribo*-hexopyranosyl-(1→3)-D-galactopyranose, have identical absolute configurations (if the configurations of the monosaccharides were different, the high-field effect on C(4) would be no smaller than 2 ppm (*cf.* Ref. 6)). Consequently, 3,6-dideoxyhexose produced upon degradation of erwiniose has the D-*ribo* configuration, *i.e.*, it is paratose.

The relative configuration of erwiniose, which was established by NMR spectroscopy, was confirmed and its absolute configuration was determined by the analysis of the periodate oxidation products of the polysaccharide. Thus, erwiniose is 3,6,8-trideoxy-4-*C*-(*R*-1-hydroxyethyl)-D-*gulo*-octose. Hitherto, this higher ten-carbon branched monosaccharide has not been found in natural carbohydrates. It should be noted that structurally similar monosaccharides, which are also branched at C(4) and

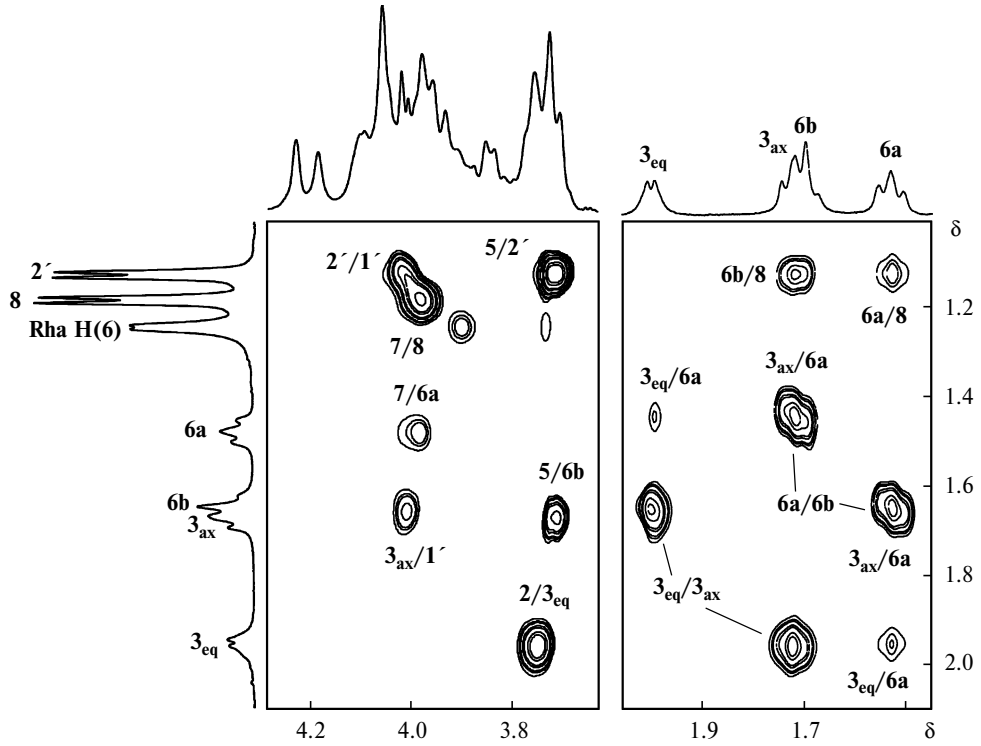


Fig. 3. Fragments of the ROESY spectrum of *O*-deacetylated polysaccharide **2**; the corresponding regions of the ^{1}H NMR spectrum are shown along the axes; the protons of erwiniose are numbered.

contain the methylene group at position 3 and the terminal methyl groups in both chains, are known. These are stereoisomeric yersinioses A and B with an eight-carbon skeleton and caryophyllose with a twelve-carbon skeleton.[7] The total structure of O-polysaccharide **1** from *E. carotovora* ssp. *atroseptica* GSPB 9205 presented in Scheme 1 is unique among the structures of bacterial polysaccharides.

Experimental

Isolation and *O*-deacetylation of the O-polysaccharide. The *E. carotovora* ssp. *atroseptica* GSPB 9205 bacterial culture from the Göttingen Collection of phytopathogenic bacteria (University of Göttingen, Germany) was grown according to a known procedure[8] in King's medium B using glycerol as a carbon source. The lipopolysaccharide was isolated by extraction with hot aqueous phenol[9] as described earlier.[1] O-Polysaccharide **1** was isolated by degradation of the lipopolysaccharide with 2% AcOH at 100 °C for 1.5 h followed by gel chromatography on a column (70×2.6 cm) with Sephadex G-50 (S) in a 0.05 *M* pyridinium acetate buffer (pH 4.5) with the use of a Knauer differential refractometer (Germany) for monitoring. Polysaccharide **1** was subjected to *O*-deacetylation by heating with 12% NH_4OH at 37 °C for 16 h.

Monosaccharide analysis and methylation analysis. *O*-Deacetylated polysaccharide **2** (0.5 mg) was hydrolyzed with

Table 4. ^{1}H NMR spectroscopic data for oligosaccharide **3**

Unit	δ (^3J/Hz)						
	H(1)	H(2)	H(3) (H$_{eq}$(3))	H(4) (H(4a))	H(5) (H(4b))	H(6) (H(6a))	H(6b)
α-D-Gal*p*-(1→ (**A**)	5.23 ($J_{1,2}$ = 4.0)	3.80 ($J_{2,3}$ = 10.2)	3.92 ($J_{3,4}$ = 3.3)	3.99 ($J_{4,5}$ < 2)	4.10	3.72	3.72
→3)-α-D-Gal*p*-(1→ (**B**)	5.29 ($J_{1,2}$ = 3.8)	4.02 ($J_{2,3}$ = 10.4)	3.98 ($J_{3,4}$ = 3.3)	4.20 ($J_{4,5}$ < 2)	4.08	3.73	3.73
→2,3)-α-D-Man*p*-(1→ (**C**)	5.35 ($J_{1,2}$ < 2)	4.18 ($J_{2,3}$ = 3.1)	4.11 ($J_{3,4}$ = 9.8)	3.98 ($J_{4,5}$ = 9.8)	3.87	3.88	3.80
→3)-1-Deoxyerythritol (**D´**)	1.19	4.02	3.64	3.79	3.72	—	—
β-Par*p*-(1→ (**E´**)*	4.60 ($J_{1,2}$ = 7.8)	3.57 ($J_{2,3_{eq}}$ = 3.5)	1.50 ($J_{2,3_{ax}}$ = 11.5)	3.38 ($J_{3_{ax},4}$ = 11.5)	3.47 ($J_{4,5}$ = 9.3)	1.26 ($J_{5,6}$ = 6.1)	—

* The following signal was also observed (δ, *J*/Hz): 2.34 (H$_{ax}$(3), $J_{3_{eq},3_{ax}}$ = 11.5, $J_{3_{eq},4}$ = 4.8).

2 *M* CF_3CO_2H at 120 °C for 2 h. Monosaccharides were transformed into alditol acetates[10] and analyzed by GLC on a Hewlett—Packard 5880 model chromatograph (USA) equipped with a capillary column with phase DB-5 using a temperature gradient from 160 °C (1 min) to 250 °C at a rate of 3 °C min^{-1}. To determine the absolute configurations, the monosaccharides were transformed into acetylated *S*-2-octyl glycosides,[11] which were analyzed by GLC under the above-described conditions.

O-Deacetylated polysaccharide **2** (1 mg) was subjected to methylation with MeI in DMSO in the presence of $CH_3SOCH_2^-$ anion.[12] After hydrolysis under the same conditions as those used in the monosaccharide analysis, partially methylated monosaccharides were transformed into alditol acetates and studied by GLC-mass spectrometry on a Hewlett—Packard 5890 model chromatograph (USA) connected to a NERMAG R10-10L mass spectrometer (France) in the above-described chromatographic conditions.

Periodate oxidation and Smith degradation. *O*-Deacetylated polysaccharide **2** (15 mg) was oxidized with 0.05 *M* $NaIO_4$ (3 mL) at 4 °C in the dark for 64 h. After deionization with CG120 cation-exchange resin (H^+ form) and IRA402 anion-exchange resin (OH^- form), 3-hydroxybutyraldehyde and a polysaccharide were isolated by gel chromatography on a column (90×2.5 cm) with TSK HW-40 in water. The aldehyde was oxidized with bromine (50 μL in 1 mL of water, 20 °C, 16 h). The mixture was concentrated and heated with *S*-octan-2-ol (150 μL) in the presence of CF_3CO_2H (15 μL) at 100 °C for 4 h. The reagents were removed with a stream of air. The residue was acylated with $(CF_3CO)_2O$ (4 °C, 16 h) and analyzed by GLC and compared with the corresponding derivatives of the authentic samples of *R*- and *S*-3-hydroxybutyric acids. The oxidized polysaccharide was reduced with $NaBH_4$ in water (20 °C, 16 h). After acidification with AcOH to pH 5, the polysaccharide was isolated by gel chromatography on TSK HW-40. Then the polysaccharide was hydrolyzed with 4% AcOH (105 °C, 2 h). Chromatography of the hydrolyzate on the same gel afforded oligosaccharide **3**.

NMR spectroscopy. Samples for spectroscopic studies were lyophilized twice from 99.9% D_2O, and the spectra were recorded for solutions in 99.96% D_2O on a Bruker DRX-500 spectrometer (Germany) at 35 °C for polysaccharides or at 30 °C for oligosaccharide **3**. The signal of HDO was suppressed by presaturation for 1 s. The data were collected and processed using the XWINNMR 3.1 software. The chemical shifts are given relative to sodium 3-trimethylsilylpropionate (δ_H 0) or acetone (δ_C 31.45). In the TOCSY and ROESY experiments, the mixing time was 200 and 100 ms, respectively. Other parameters do not differ significantly from those described earlier.[13]

This study was financially supported by the Council on Grants of the President of the Russian Federation (Program for State Support of Young Scientists and Leading Scientific Schools of the Russian Federation, Grant NSh-1557.2003.3) and the Russian Foundation for Basic Research (Project No. 02-04-48721).

References

1. S. N. Senchenkova, Y. A. Knirel, A. S. Shashkov, M. Ahmed, A. Mavridis, and K. Rudolph, *Carbohydr. Res.*, 2003, **338**, 2025.
2. S. Fukuoka, Y. A. Knirel, B. Lindner, H. Moll, U. Seydel, and U. Zähringer, *Eur. J. Biochem.*, 1997, **250**, 55.
3. S. Fukuoka, H. Kamishima, Y. Nagawa, H. Nakanishi, K. Ishikawa, Y. Niwa, E. Tamiya, and I. Karube, *Arch. Microbiol.*, 1992, **157**, 311.
4. T. C. Ray, A. R. W. Smith, R. Wait, and R. C. Hignet, *Eur. J. Biochem.*, 1987, **170**, 357.
5. R. Sandulache and P. Prehm, *J. Bacteriol.*, 1985, **161**, 1226.
6. A. S. Shashkov, G. M. Lipkind, Y. A. Knirel, and N. K. Kochetkov, *Magn. Reson. Chem.*, 1988, **26**, 735.
7. N. K. Kochetkov, *Usp. Khim.*, 1996, **65**, 799 [*Russ. Chem. Rev.*, 1996, **65** (Engl. Transl.)].
8. S. N. Senchenkova, X. Huang, P. Laux, Y. A. Knirel, A. S. Shashkov, and K. Rudolph, *Carbohydr. Res.*, 2002, **337**, 1723.
9. O. Westphal and K. Jann, *Methods Carbohydr. Chem.*, 1965, **5**, 83.
10. J. S. Sawardeker, J. H. Sloneker, and A. Jeanes, *Anal. Chem.*, 1965, **37**, 1602.
11. K. Leontein and J. Lönngren, *Methods Carbohydr. Chem.*, 1993, **9**, 87.
12. H. E. Conrad, *Methods Carbohydr. Chem.*, 1972, **6**, 361.
13. O. Hanniffy, A. S. Shashkov, S. N. Senchenkova, S. V. Tomshich, N. A. Komandrova, L. A. Romanenko, Y. A. Knirel, and A. V. Savage, *Carbohydr. Res.*, 1999, **321**, 132.

Received April 11, 2005;
in revised form April 18, 2005