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Note

Structural heterogeneity in the lipopolysaccharides of *Pseudomonas syringae* with O-polysaccharide chains having different repeating units

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Abstract

Studies by sugar and methylation analyses, Smith degradation, and ^{1}H and ^{13}C NMR spectroscopy revealed a structural heterogeneity in the O-polysaccharides of *Pseudomonas syringae* pvs. coronafaciens IMV 9030 and atrofaciens IMV 8281 owing to the presence of different types of repeating units. In strain IMV 9030, the major repeating units are a linear α-L-rhamnose trisaccharide and a tetrasaccharide (**A**, $n = 0$ or 1). A minor repeating unit is a branched pentasaccharide with an α-L-rhamnose main chain and a lateral 3-acetamido-3,6-dideoxy-D-galactose (D-Fuc3NAc) residue (**B**, X = 2, $n = 1$). In strain IMV 8281, all repeating units are branched and differ in size and position of substitution of one of the α-L-rhamnose residues (tetrasaccharide, **B**, X = 3, $n = 0$; pentasaccharides, **B**, X = 2 or 3, $n = 1$).

→2)-α-L-Rha*p*-(1-[→2)-α-L-Rha*p*-(1-]$_n$→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→ **A**

→2)-α-L-Rha*p*-(1→X)-α-L-Rha*p*-(1-[→3)-α-L-Rha*p*-(1-]$_n$→3)-α-L-Rha*p*-(1→ **B**

3
↑
1
α-D-Fuc*p*3NAc

Reinvestigation of the structure of the branched O-polysaccharide of *P. syringae* pv. tomato IPGR 140 showed that, together with the major tetrasaccharide repeating unit (**B**, X = 3, $n = 0$) [Knirel, Y. A., et al. *Carbohydr. Res.* **1993**, *243*, 199–204], it has a minor pentasaccharide repeating unit (**B**, X = 3, $n = 1$). © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Lipopolysaccharide; O-Antigen; Bacterial polysaccharide; Structural heterogeneity; Phytopathogen; *Pseudomonas syringae*

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Pseudomonas syringae and related species occur worldwide and are important plant pathogens. According to the host specificity, they are divided into infraspecies taxa, so called pathovars. Recently, structures of the *O*-specific polysaccharide chains of the lipopolysaccharides of a number of *P. syringae* strains belonging to various pathovars have been established [Refs. 1–3 and references cited therein]. A peculiar feature of the polysaccharides is the presence of a rhamnan backbone composed of L-, D-, or both L- and D-rhamnose residues. The polysaccharides that contain four L-rhamnose residues in the repeating unit lack a strict regularity owing to a different mode of substitution of one of the rhamnose residues. In the present paper, we report on structural heterogeneity in *P. syringae* lipopolysaccharides which is associated with the presence in one strain of both linear and branched O-polysaccharides and a variation in the number of L-rhamnose residues in the repeating unit.

The lipopolysaccharides of *P. syringae* pv. coronafaciens IMV 9030 and atrofaciens IMV 8281 were isolated from bacterial cells by extraction with saline.[3] The O-polysaccharides were obtained by mild-acid degradation of the lipopolysaccharides followed by GPC on Sephadex G-50. Sugar analysis by GLC of the alditol acetates obtained after acid hydrolysis of the polysaccharides revealed rhamnose and 3-amino-3,6-dideoxygalactose (Fuc3N) in the ratio 12.2:1 and 3.4:1 (detector response data), respectively. In addition, a trace amount of glucose was detected, which, most likely, originated from the lipopolysaccharide core.[4] GLC of the acetylated glycosides with chiral alcohols showed that, in both polysaccharides, rhamnose has the L configuration and Fuc3N has the D configuration. Methylation analysis[5] showed the presence of 2-substituted Rha, 3-substituted Rha, 2,3-disubstituted Rha, and terminal Fuc3N in the ratios 5.6:9.2:1:0.5 and 0.7:1.7:1:0.3 in the polysaccharides of strains IMV 9030 and IMV 8281, respectively, as well as minor components from the lipopolysaccharide core.

P. syringae *pv. coronafaciens IMV 9030.*— The ^{1}H and ^{13}C NMR spectra of the polysaccharide (Figs. 1(a) and 2(a)) contained signals of different intensities, thus showing the lack of a strict regularity or the presence of a polysaccharide mixture. Assignment of signals of the major series using 2D COSY, TOCSY, and H-detected $^{1}H,^{13}C$ HMQC experiments (Tables 1 and 2) showed that they belonged to a linear rhamnan having a trisaccharide repeating unit of structure **1**. In particular, the α configuration of all glycosidic linkages followed from the chemical shifts δ_H 3.75–3.86 for H-5 and δ_C 70.4–70.5 for C-5 (compare the H-5 chemical shift δ 3.86 in α-Rha but δ 3.39 in β-Rha,[6] and the C-5 chemical shift δ 70.0 in α-Rha*p* but δ 72.3 in β-Rha*p*[7]). The substitution pattern and sugar sequence were determined by a low-field position at δ_C 79.0–79.2 of the signals for C-3 of two Rha residues (Rha^{I} and Rha^{II}) and C-2 of the third Rha residue (Rha^{III}), as compared with their position in non-substituted α-Rha*p* at δ_C 71.3–71.6.[7] The structure **1** was confirmed by a NOESY experiment, which revealed interresidue Rha^{III} H-1/Rhap^{II} H-3, Rha^{II} H-1/Rhap^{I} H-3, and Rha^{I} H-1/Rhap^{III} H-2 correlations at δ 5.19/3.89, 5.03/3.83, and 4.96/4.07, respectively.

→2)-α-L-Rhap^{III}-(1→3)-α-L-Rhap^{II}-(1→3)-α-L-Rhap^{I}-(1→ **1**

A minor series of signals in the ^{13}C NMR spectrum (Fig. 2(a)) belonged to a branched polysaccharide containing Rha and Fuc3NAc residues. It included signals for C-1–C-6 of a nonsubstituted α-Fuc3NAc residue at δ 97.0, 67.1, 52.4, 71.6, 68.1, and 16.4, those for C-2–C-4 of a 2,3-disubstituted Rha residue (Rha^{IV}) at δ 75.8, 77.0, and 72.2, respectively, and a signal for C-1 of a Rha residue (Rha^{I}) linked to Rha^{IV} at δ 102.6. The chemical shifts of these signals were practically identical to those in the major O-polysaccharide of *P. syringae* pvs. syringae IMV 281[8] and atrofaciens IMV 8281 (see below) having the structure **2** (Table 2). 2D COSY, TOCSY, NOESY, and $^{1}H,^{13}C$ HMQC experiments confirmed the presence of other signals for structure **2**, most of which in the 1D ^{1}H and ^{13}C NMR spectra coincided with major signals.

Tracing connectivities for the remaining signals having an intermediate intensity in the 2D COSY and TOCSY spectra suggested that

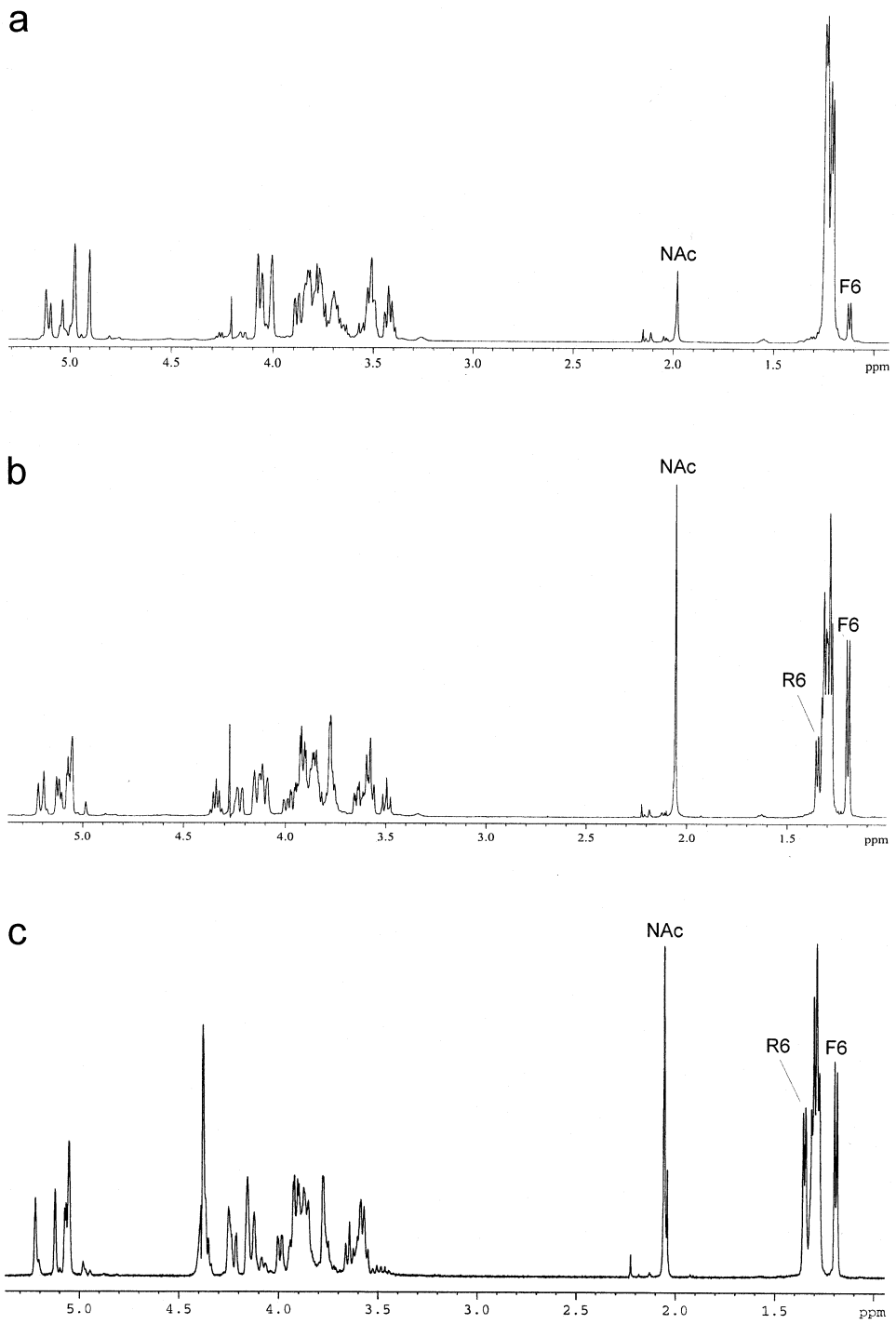


Fig. 1. 500-MHz ^{1}H NMR spectra of the O-polysaccharides of *P. syringae* pv. coronafaciens IMV 9030 (a) and pv. atrofaciens IMV 8281 (b), and the Smith-degraded polysaccharide of *P. syringae* pv. atrofaciens IMV 8281 (c). Roman numerals refer to carbons in sugar residues designated as F for Fuc3NAc and R for Rha^{I} (R1) and Rha^{IV} (R2–R4) in **2** (a), Rha^{IV} in **2** and Rha^{III} in **6** and **7** (b), or Rha^{I} in **6** and **7** (c); designations for Fuc3NAc and Rha^{III} in **6** and **7** (b) are italicised.

they belonged to another linear structure **3** with a tetrasaccharide repeating unit. The positions of these signals were essentially identical to those of the O-polysaccharide of *P. syringae* pv. atrofaciens IMV 7836[2] that has the same linear structure but consists of D-Rha (**4**). For instance, the signals for H-1–H-5 of the fourth rhamnose residue (Rha^{IV}) in the

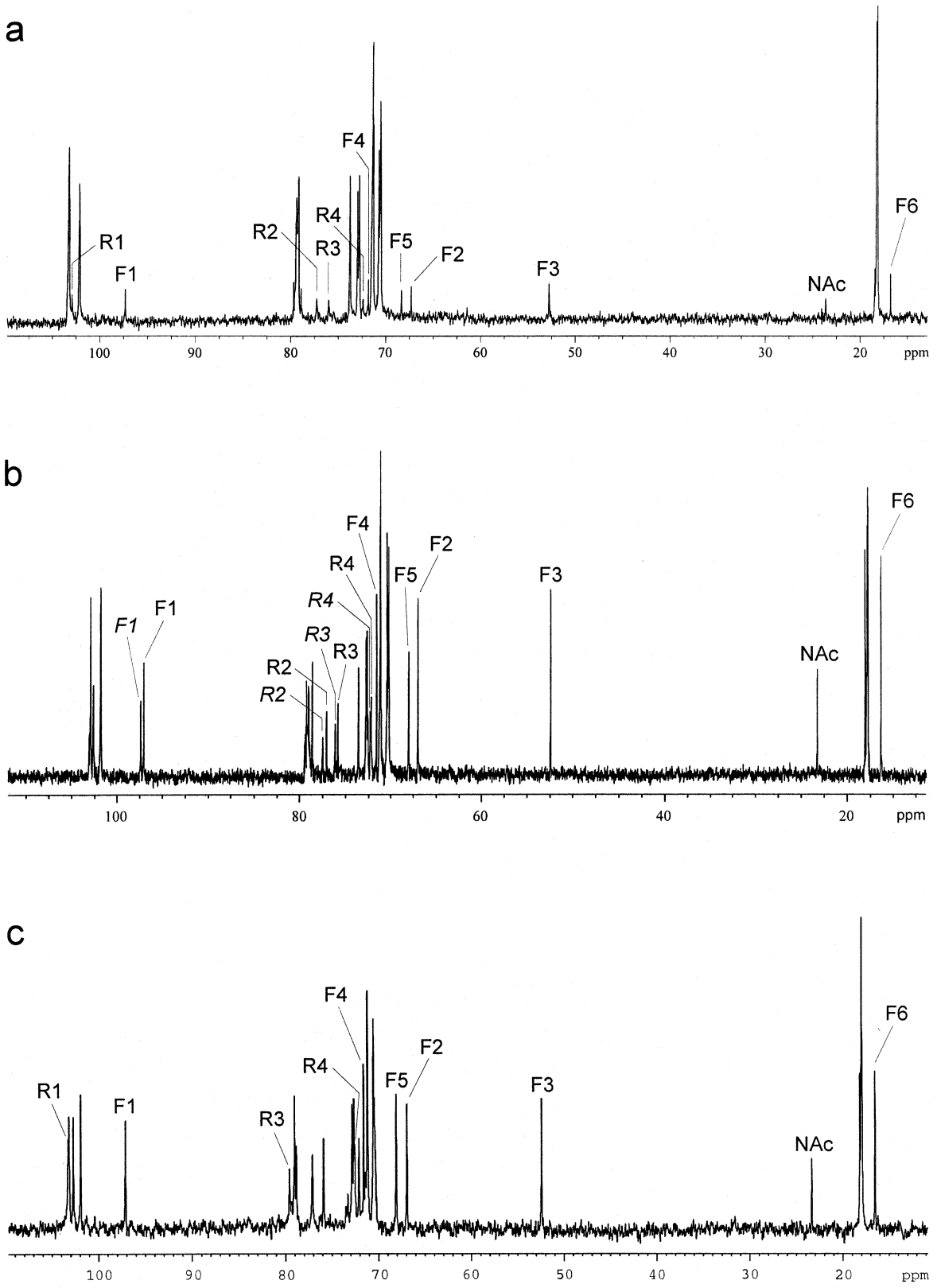
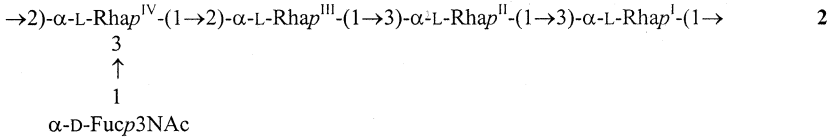


Fig. 2. 125-MHz ^{13}C NMR spectra of the O-polysaccharides of *P. syringae* pv. coronafaciens IMV 9030 (a) and pv. atrofaciens IMV 8281 (b), and the Smith-degraded polysaccharide of *P. syringae* pv. atrofaciens IMV 8281 (c). Designations R6 and F6 refer to Rha^{III} H-6 in **6** and **7** (b,c) and Fuc3NAc H-6, respectively.

Table 1
^{1}H NMR chemical shifts (δ in ppm)

Sugar residue	H-1	H-2	H-3	H-4	H-5	H-6
O-Polysaccharide of P. syringae *pv. coronafaciens IMV 9030*						
Structure ***1***						
→3)-α-L-Rha*p*^{I}-(1→	4.96	4.14	3.83	3.57	3.75	1.27
→3)-α-L-Rha*p*^{II}-(1→	5.03	4.12	3.89	3.57	3.86	1.30
→2)-α-L-Rha*p*^{III}-(1→	5.19	4.07	3.94	3.48	3.82	1.30
O-Polysaccharide of P. syringae *pv. atrofaciens IMV 7836*[2]						
Structure ***4***						
→3)-α-L-Rha*p*^{I}-(1→	4.96	4.15	3.83	3.56	3.76	1.27
→3)-α-L-Rha*p*^{II}-(1→	5.03	4.12	3.90	3.57	3.87	1.27
→2)-α-L-Rha*p*^{III}-(1→	5.17	4.07	3.95	3.49	3.82	1.30
→2)-α-L-Rha*p*^{IV}-(1→	5.11	4.08	3.89	3.48	3.72	1.30
O-Polysaccharide of P. syringae *pv. atrofaciens IMV 8281*						
Structure ***6***						
→3)-α-L-Rha*p*^{I}-(1→	5.02	4.17	3.91	3.58	3.87 [a]	1.31 [a]
→3)-α-L-Rha*p*^{II}-(1→	5.03	4.17	3.94	3.57	3.88 [a]	1.30 [a]
→2,3)-α-L-Rha*p*^{III}-(1→	5.24	4.26	3.99	3.65	3.89	1.35
→3)-α-L-Rha*p*^{IV}-(1→	5.14	4.15	3.86	3.54	3.76	1.28
α-D-Fuc*p*3NAc-(1→	5.07	3.92	4.23	3.77	4.39	1.18
Structure ***7***						
→3)-α-L-Rha*p*^{I}-(1→	5.14	4.14	3.85	3.54	3.76	1.28
→3)-α-L-Rha*p*^{II}-(1→	5.02	4.17	3.91	3.58	3.87 [a]	1.31 [a]
→2,3)-α-L-Rha*p*^{III}-(1→	5.24	4.26	3.99	3.65	3.89	1.35
α-D-Fuc*p*3NAc-(1→	5.07	3.92	4.23	3.77	4.39	1.18

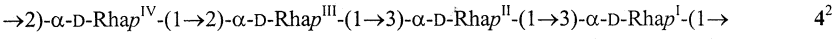
Chemical shift for NAc is δ 2.04–2.06.
[a] Assignment could be interchanged.



2

structure **3** were at δ 5.11, 4.08, 3.88, 3.47 and 3.71, and those for H-1 and H-2 of Rha^{III} were at δ 5.17 and 4.07, respectively (compare the data of the structure **4**, Table 1). The other ^{1}H NMR signals and the most ^{13}C NMR signals of the structure **3** coincided with those of the structure **1**. The correlation pattern in the NOESY spectrum was consistent with the structure **3**.

3



4[2]

Structure **3** differs from structure **1** by the presence of one additional 2-substituted Rha residue (Rha^{IV}) and is identical to the backbone structure of the branched polysaccharide **2**. It remains unknown whether repeating units of different structures are present in the same or different polysaccharide chains. As judged by relative intensities of signals in the NMR spectra, structures **1**, **2**, and **3** are present in *P. syringae* pv. coronafaciens IMV 9030 in the ratios ~11:3:6. Structure **1** has not been previously reported in *P. syringae* lipopolysaccharides, whereas the branched structure **2** is identical to the major O-polysaccharide structure in *P. syringae* pv. syringae IMV 281,[8] and the linear structure **3** to a minor O-polysaccharide structure in *P. syringae* pv. garcae NCPPB 2708.[9]

Table 2
^{13}C NMR chemical shifts (δ in ppm)

Sugar residue	C-1	C-2	C-3	C-4	C-5	C-6
O-Polysaccharide of P. syringae *pv. coronafaciens IMV 9030*						
Structure ***1***						
→3)-α-L-Rha*p*^{I}-(1→	103.0	71.1	79.0	72.8	70.5	17.8 [a]
→3)-α-L-Rha*p*^{II}-(1→	103.0	71.2	79.1	72.6	70.4	17.9 [a]
→2)-α-L-Rha*p*^{III}-(1→	101.9	79.2	71.2	73.6	70.4	17.8 [a]
O-Polysaccharide of P. syringae *pv. atrofaciens IMV 8281*						
Structure ***2***						
→3)-α-L-Rha*p*^{I}-(1→	102.8	71.3	78.7	72.8 [a]	70.6	17.9
→3)-α-L-Rha*p*^{II}-(1→	103.0	71.3	79.2	72.7 [a]	70.6 [a]	17.9
→2)-α-L-Rha*p*^{III}-(1→	102.0	79.4	71.4	73.7	70.4	17.9
→2,3)-α-L-Rha*p*^{IV}-(1→	102.0	75.9	77.1	72.3	70.4 [a]	18.1
α-D-Fuc*p*3NAc-(1→	97.1	67.1	52.5	71.7	68.1	16.4
Structure ***6***						
→3)-α-L-Rha*p*^{I}-(1→	103.5	71.3	79.7	72.7	70.6 [a]	18.0
→3)-α-L-Rha*p*^{II}-(1→	103.4	71.3	79.0 [a]	73.0	70.6 [a]	18.0
→2,3)-α-L-Rha*p*^{III}-(1→	102.1	76.1	77.3	72.2	70.6 [a]	18.2
→3)-α-L-Rha*p*^{IV}-(1→	102.9	71.3	79.2 [a]	72.8	70.5	18.0
α-D-Fuc*p*3NAc-(1→	97.3	67.1	52.5	71.7	68.2	16.5
Structure ***7***						
→3)-α-L-Rha*p*^{I}-(1→	102.9	71.3	79.2 [a]	72.8	70.5	18.0
→3)-α-L-Rha*p*^{II}-(1→	103.4	71.3	79.2 [a]	73.0	70.5 [a]	18.0
→2,3)-α-L-Rha*p*^{III}-(1→	102.1	76.1	77.3	72.2	70.6 [a]	18.2
α-D-Fuc*p*3NAc-(1→	97.3	67.1	52.5	71.7	68.2	16.5

Chemical shifts for NAc are δ 23.2–23.4 (CH_3) and 175.4–175.7 (CO).
[a] Assignment could be interchanged.

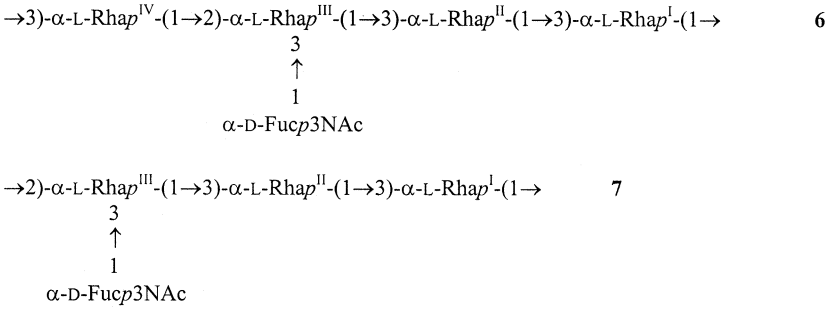
P. syringae *pv. atrofaciens IMV 8281.*—The ^{1}H and ^{13}C NMR spectra of the polysaccharide (Figs. 1(b) and 2(b)) indicated a structural heterogeneity again. The ^{13}C NMR spectrum showed, inter alia, signals for anomeric carbons at δ 97.1–103.0, one carbon bearing nitrogen (C-3 of Fuc3N) at δ 52.5, and one *N*-acetyl group (Me at δ 23.4, CO at δ 175.7).

The chemical shifts for signals of the major series in the ^{13}C NMR spectrum (Table 2) and the corresponding cross-peaks in the ^{1}H,^{13}C HMQC spectrum were essentially identical to those of the major polysaccharide of *P. syringae* pv. syringae IMV 281[8] and a minor polysaccharide of *P. syringae* pv. coronafaciens IMV 9030 (see above) both having structure **2**. This structure was confirmed by Smith degradation of the O-polysaccharide, which gave an oligosaccharide and a polysaccharide. NMR spectroscopic studies showed that the oligosaccharide contains one Fuc3NAc, one glycerol, and three Rha residues and is identical to the oligosaccharide **5** derived by Smith degradation from the major polysaccharide **2** of *P. syringae* pv. syringae IMV 281[8] as a result of oxidation of Rha^{III}.

α-L-Rha*p*^{II}-(1→3)-α-L-Rha*p*^{I}-(1→2)-α-L-Rha*p*^{IV}-(1→2)-Gro **5**
3
↑
1
α-D-Fuc*p*3NAc

The ^{1}H and ^{13}C NMR spectra of the polysaccharide obtained by Smith degradation (Figs. 1(c) and 2(c)) confirmed the presence of structure(s) closely related to **2**. Comparison with published data[8,10,11] suggested that the ^{13}C NMR spectrum is a superposition of the spectra for **6** (major) and **7** (minor) characterised by repeating units with one α-Fuc*p*3NAc and four or three α-Rha*p* residues, respectively. The ^{1}H NMR spectrum (Fig. 1(c)) showed, inter alia, a significant increase in the intensity of the signal at δ 1.35 as compared to the spectrum of the initial polysaccharide (Fig. 1(b)). This signal belonged to H-6 of the

disubstituted rhamnose residue (Rha^{III}) in the structures **6** and **7** (Table 1) and may serve as a marker to distinguish these structures from **2**.



6

7

The assignment of the ^{1}H and ^{13}C NMR spectra using COSY, TOCSY, and $^{1}H,^{13}C$ HMQC experiments (Tables 1 and 2) showed that the major signals for **7** coincided with the corresponding signals for **6**, but signals for C-1, C-3, and C-4 of Rha^{I} from **6** resonated separately at δ 103.5, 79.7, and 72.7, respectively. The two structures could be distinguished also by different correlation patterns between the anomeric and linkage protons in the NOESY spectrum (compare correlations Rha^{III} H-1,Rha^{II} H-3; Rha^{II} H-1,Rha^{I} H-3; Rha^{I} H-1,Rha^{IV} H-3; Rha^{IV} H-1,Rha^{I} H-2; and Fuc3NAc H-1,Rha^{III} H-3 at δ 5.24/3.94; 5.03/3.91; 5.02/3.86; 5.14/4.26; and 5.07/3.99 in the major series and Rha^{III} H-1,Rha^{II} H-3; Rha^{II} H-1,Rha^{I} H-3; Rha^{I} H-1,Rha^{III} H-2; and Fuc3NAc H-1,Rha^{III} H-3 at δ 5.24/3.91; 5.02/3.85; 5.14/4.26; and 5.07/3.99 in the minor series, respectively).

Based on the signal intensities in the ^{13}C and ^{1}H NMR spectra of the initial and Smith-degraded polysaccharides, it was concluded that in *P. syringae* pv. atrofaciens IMV 8281 the repeating units **2**, **6**, and **7** are present in the ratios ~13:5:2, respectively. Not only **2** (see above), but also **6** and **7** have been previously reported: the former is a minor O-polysaccharide component in *P. syringae* pvs. syringae IMV 281[8] and holci (syringae) IMV 8300[10] and the latter is the major component in *P. syringae* pv. tomato IPGR 140[11] (see also below).

Therefore, the heterogeneity in the structure of the *O*-specific polysaccharide of *P. syringae* pv. atrofaciens IMV 8281 is associated with variations in the number of rhamnose residues in the repeating unit and in the position of substitution of one of these residues. The heterogeneity of the second type has been previously found in *P. syringae* O-polysaccharides that contain an L-rhamnan backbone with four Rha residues in the backbone.[3,8–10,12,13] The heterogeneity of the first type has not been hitherto reported. Analysis of published data showed that, most likely, in addition to the pentasaccharide repeating units,[8,10] the O-polysaccharides of *P. syringae* pvs. syringae IMV 281 and holci (syringae) IMV 8300 contain minor repeating units of **7** and thus are also characterised by the heterogeneity of both types. Contrariwise, reinvestigation of the O-polysaccharide of *P. syringae* tomato IPGR 140 using 2D NMR spectroscopy revealed the presence of not only **7**, which was reported previously,[11] but also **6** in a ~2:1 ratio.

1. Experimental

Cultivation of bacteria, isolation and degradation of lipopolysaccharides.—Strains of *P. syringae* from the collection of the D.K. Zabolotny Institute of Microbiology and Virology were grown on a potato agar at 26–28 °C for 20–22 h. Lipopolysaccharides were isolated by extraction of bacterial cells with 0.85% saline and purified by ultracentrifugation (105,000*g*, 3 h). O-Polysaccharides were obtained by degradation of the lipopolysaccharides with aq 2% HOAc for 1.5 h at 100 °C followed by GPC on a column (56 × 2.6 cm) of Sephadex G-50 (S) (Pharmacia) using 0.05 M pyridine acetate (pH 4.5) as eluent and monitoring with a Knauer differential refractometer.

Sugar and methylation analysis.—Hydrolysis was performed with 2 M CF_3CO_2H

(120 °C, 2 h). Monosaccharides were identified by GLC as their alditol acetates on an Ultra 2 capillary column using a Hewlett–Packard 5880 instrument and a temperature gradient of 160 °C (1 min) to 290 °C at 10 °C/min. The absolute configurations were determined by GLC of the acetylated glycosides with (*S*)-2-octanol (for Rha) and (*S*)-2-butanol (for Fuc3N).[14–16]

Methylation was carried out with MeI in dimethyl sulfoxide in the presence of solid NaOH.[5] Hydrolysis was performed as for sugar analysis: partially methylated monosaccharides were converted into the alditol acetates and analysed by GLC–MS using a Hewlett–Packard 5989A instrument equipped with an HP-5 column and a temperature gradient 150 °C (3 min) → 320 °C at 5 °C/min.

Smith degradation.—OPS of *P. syringae* pv. atrofaciens IMV 8281 (20 mg) was oxidised with 0.1 M $NaIO_4$ in dark for 48 h at 20 °C. After reduction with $NaBH_4$ and desalting on a column (80 × 1.6 cm) of TSK HW-40 (S) (E. Merck, Germany) in aq 1% HOAc, the product was hydrolysed with aq 2% HOAc for 2 h at 100 °C, reduced with $NaBH_4$ and, after treatment with a KU-2 (H^+-form) cation-exchange resin, fractionated on TSK HW-40 (S) to give a polysaccharide (4.3 mg), an oligosaccharide (4.2 mg), and two intermediate fractions (4.9 and 0.9 mg).

NMR spectroscopy.—Samples were deuterium-exchanged by freeze-drying from ^2H_2O. ^1H and ^{13}C NMR spectra were recorded with a Bruker DRX-500 spectrometer for solutions in ^2H_2O at 60 °C for the starting polysaccharide, 12 °C for the Smith-degraded polysaccharide, and 30 °C for the oligosaccharide-glycerol **5**. Chemical shifts are reported with internal acetone (δ_H 2.225, δ_C 31.45). A mixing time of 200 and 150 ms was used in TOCSY and NOESY experiments, respectively.

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References

1. Knirel, Y. A.; Zdorovenko, G. M. In *Pseudomonas syringae Pathovars and Related Pathogens*; Rudolph, K.; Burr, T. J.; Mansfield, J. W.; Stead, D.; Vivian, A.; von Kietzell, J., Eds.; Kluwer Academic: Dordrecht, Boston, London, 1997; pp. 475–480.
2. Ovod, V.; Knirel, Y. A.; Samson, R.; Krohn, K. *J. Bacteriol.* **1999**, *181*, 6937–6947.
3. Zdorovenko, G. M.; Shashkov, A. S.; Zdorovenko, E. L.; Kocharova, N. A.; Yakovleva, L. M.; Knirel, Y. A.; Rudolph, K. *Biochemistry (Moscow)* **2001**, *66*, 369–377.
4. Zdorovenko, G. M.; Gubanova, N. Y.; Solyanik, L. P.; Knirel, Y. A.; Yakovleva, L. M.; Zakharova, I. Y. In *Pseudomonas syringae Pathovars*, Proceedings of the 5th International Working Group, Florence, Italy, 1991; pp. 391–401.
5. Ciucanu, I.; Kerek, F. *Carbohydr. Res.* **1984**, *131*, 209–217.
6. Jansson, P.-E.; Kenne, L.; Widmalm, G. *Carbohydr. Res.* **1989**, *188*, 169–191.
7. Lipkind, G. M.; Shashkov, A. S.; Knirel, Y. A.; Vinogradov, E. V.; Kochetkov, N. K. *Carbohydr. Res.* **1988**, *175*, 59–75.
8. Knirel, Y. A.; Zdorovenko, G. M.; Shashkov, A. S.; Mamyan, S. S.; Gubanova, N. Y.; Yakovleva, L. M.; Solyanik, L. P. *Bioorg. Khim.* **1988**, *14*, 180–186.
9. Zdorovenko, E. L.; Knirel, Y. A.; Ovod, V. V. In *Abstract Book of the 13th International Congress of the Hungarian Society of Microbiology*, Budapest, Hungary, 1999; p. 112.
10. Knirel, Y. A.; Zdorovenko, G. M.; Shashkov, A. S.; Yakovleva, L. M.; Gubanova, N. Y.; Gvozdyak, R. I. *Bioorg. Khim.* **1988**, *14*, 172–179.
11. Knirel, Y. A.; Shashkov, A. S.; Paramonov, N. A.; Zdorovenko, G. M.; Solyanik, L. P.; Yakovleva, L. M. *Carbohydr. Res.* **1993**, *243*, 199–204.
12. Zdorovenko, E. L.; Ovod, V.; Shashkov, A. S.; Kocharova, N. A.; Knirel, Y. A.; Krohn, K. *Biochemistry (Moscow)* **1999**, *64*, 765–773.
13. Ovod, V.; Zdorovenko, E. L.; Shashkov, A. S.; Kocharova, N. A.; Knirel, Y. A. *Eur. J. Biochem.* **2000**, *267*, 2372–2379.
14. Leontein, K.; Lindberg, B.; Lönngren, J. *Carbohydr. Res.* **1978**, *62*, 359–362.
15. Gerwig, G. J.; Kamerling, J. P.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1979**, *77*, 1–7.
16. Shashkov, A. S.; Senchenkova, S. N.; Nazarenko, E. L.; Zubkov, V. A.; Gorshkova, N. M.; Knirel, Y. A.; Gorshkova, R. P. *Carbohydr. Res.* **1997**, *303*, 333–338.