Carbohydrate Research, 243 (1993) 199–204
Elsevier Science Publishers B.V., Amsterdam

Note

Somatic antigens of pseudomonads: structure of the O-specific polysaccharide of *Pseudomonas syringae* pv. *tomato* 140(R)

Yuriy A. Knirel [a], Alexander S. Shashkov [a], Nikolay A. Paramonov [a], Galina M. Zdorovenko [b], Lyudmila P. Solyanic [b] and Lyudmila M. Yakovleva [b]

[a] *N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky Pr. 47, Moscow B-334 (Russian Federation)*
[b] *D.K. Zabolotny Institute of Microbiology and Virology, Ukrainian Academy of Sciences, Ul. Zabolotnogo 156, Kiev-143 (Ukraine)*

(Received March 18th, 1992; accepted August 18th, 1992)

This work is a continuation of a chemical and immunochemical study of lipopolysaccharides of the phytopathogenic bacteria *Pseudomonas syringae* and related species (refs. 1–3 and refs. therein). We now report the structure of the O-specific polysaccharide of *P. syringae* pv. *tomato* 140(R).

The O-specific polysaccharide was obtained by mild acid degradation of the lipopolysaccharide, isolated by washing of bacterial cells with saline[4]. It had $[\alpha]_D$ +17° (*c* 0.5).

Acid hydrolysis of the polysaccharide revealed the presence of rhamnose and 3-amino-3,6-dideoxygalactose (Fuc3N), which were identified by using GLC of alditol acetates and an amino acid analyzer, respectively. GLC of (+)-2-octyl glycosides of rhamnose, as compared with (+)- and (−)-2-octyl glycosides of the authentic sample of L-rhamnose, proved[5] that this sugar has the L configuration. The D configuration of Fuc3N was established on the basis of NOE and ^{13}C chemical shift data (see below).

The ^{13}C NMR spectrum of the polysaccharide (Fig. 1, Table I) contained the signals for four anomeric carbons at 97.5–103.2 ppm, one carbon bearing nitrogen at 52.6 ppm, four CH_3–C groups (C-6 of 6-deoxyhexoses) at 16.5 (one) and 18.0–18.2 ppm, other sugar carbons in the region 67.2–79.2 ppm, and one *N*-acetyl group (Me at 23.4 ppm, CO at 175.6 ppm). In the spectrum, there were no lines characteristic of furanose rings and, hence, the constituent sugars were pyranoid.

Correspondence to: Dr. Yu.A. Knirel, N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky Pr. 47, Moscow B-334, Russian Federation.

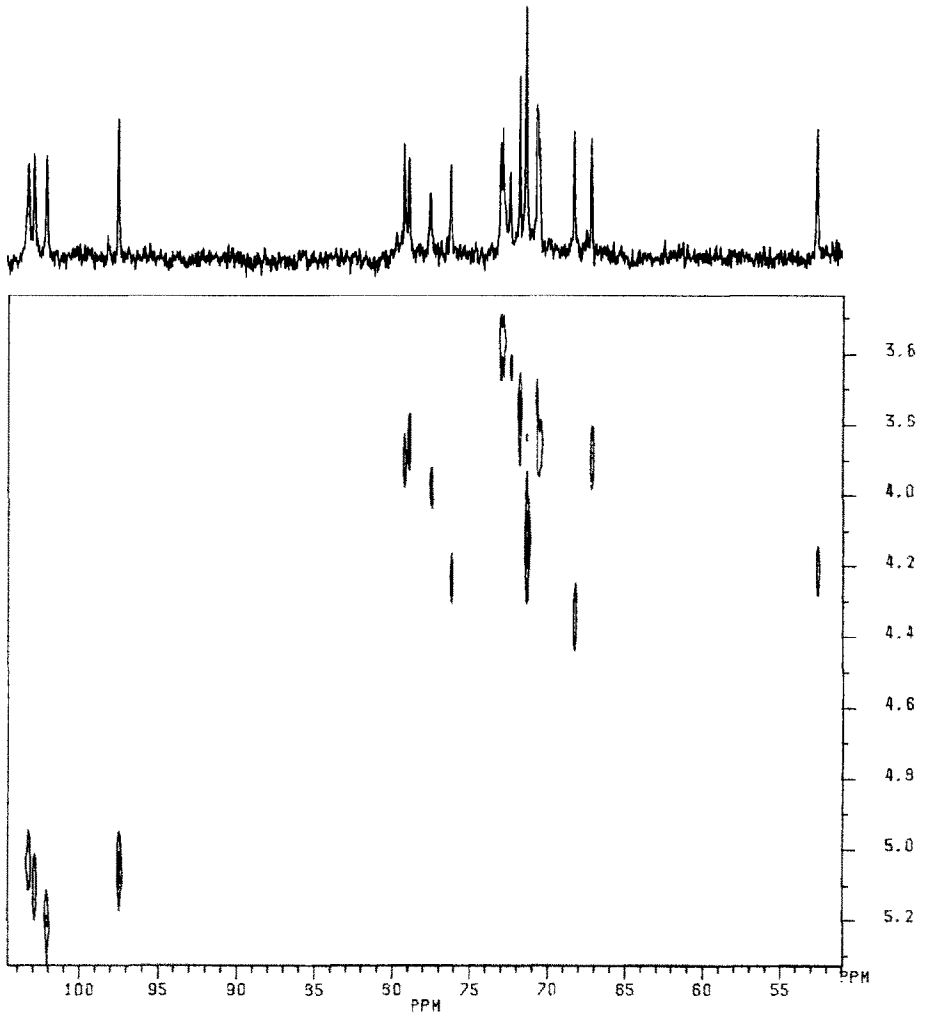

Fig. 1. 75-MHz 2D heteronuclear $^{13}C-^{1}H$ shift-correlated spectrum of the O-specific polysaccharide. The respective part of a 1D ^{13}C NMR spectrum is displayed along the F_2 axis.

TABLE I

^{13}C NMR data for the O-specific polysaccharide (δ in ppm) [a]

Unit		C-1	C-2	C-3	C-4	C-5	C-6
→ 3)-α-L-Rha*p*-(1 →	**(A)**	102.9	71.4	78.9	73.0 [b]	70.7	18.0 [d]
→ 2)-α-L-Rha*p*-(1 →	**(B)**	102.0	76.2	77.5	72.4	70.6 [c]	18.2 [d]
3 ↑							
→ 3)-α-L-Rha*p*-(1 →	**(C)**	103.2	71.4	79.2	72.9 [b]	70.5 [c]	18.0 [d]
α-D-Fuc*p*3NAc-(1 →	**(D)**	97.5	67.2	52.6	71.8	68.3	16.5

[a] Additional signals: NAc at 23.4 ppm (Me) and 175.6 ppm (CO). [b,c,d] Assignments could be interchanged.

TABLE II

^{1}H NMR data for the O-specific polysaccharide (δ in ppm, J in Hz) [a]

H-1	H-2	H-3	H-4	H-5	H-6
→3)-α-L-*Rha*p-(1→ (*unit* **A**)					
5.10	4.10	3.83	3.53	3.73	1.24
$J_{1,2}$ < 2	$J_{2,3}$ ~ 3	$J_{3,4}$ ~ 10	$J_{4,5}$ ~ 10	$J_{5,6}$ 6.5	
→2)-α-L-*Rha*p-(1→ (*unit* **B**)					
3 ↑					
5.19	4.22	3.96	3.61	3.84	1.32
$J_{1,2}$ < 2	$J_{2,3}$ 2.5	$J_{3,4}$ 9.6	$J_{4,5}$ ~ 10	$J_{5,6}$ 6.1	
→3)-α-L-*Rha*p-(1→ (*unit* **C**)					
5.01	4.13	3.89	3.55	3.84	1.26
$J_{1,2}$ < 2	$J_{2,3}$ ~ 3	$J_{3,4}$ 9.5	$J_{4,5}$ ~ 10	$J_{5,6}$ 5.9	
α-D-*Fuc*p*NAc*-(1→ (*unit* **D**)					
5.04	3.88	4.20	3.74	4.33	1.15
$J_{1,2}$ 4.0	$J_{2,3}$ ~ 11	$J_{3,4}$ 3.1	$J_{4,5}$ < 1	$J_{5,6}$ 6.6	

[a] Additional signal: NAc at 2.02 ppm.

The ^{1}H NMR spectrum of the polysaccharide (Fig. 2, Table II) contained the signals for four anomeric protons at 5.0–5.2 ppm, one of which represented a doublet with $J_{1,2}$ 4.0 Hz and belonged, thus, to H-1 of the α-linked Fuc3NAc. Three other H-1 signals were broadened singlets, and, based on their chemical shifts, one could conclude that all Rha residues are also α-linked. Four CH_3–C groups resonated at 1.1–1.35 ppm (doublets with $J_{1,2}$ 5.9–6.5 Hz), other sugar protons in the region 3.4–4.1 ppm, and the *N*-acetyl group at 2.02 ppm (s).

These data showed that the polysaccharide has a tetrasaccharide repeating unit, which includes three residues of rhamnose and one Fuc3NAc residue, all of them being in the α-pyranoid form.

The ^{1}H NMR spectrum of the polysaccharide was assigned with the use of selective spin-decoupling procedures, 2D homonuclear shift-correlated (COSY) and one-step relayed coherence transfer (COSYRCT) spectroscopy (Table II). As expected, the doublet at 5.04 ppm was identified as belonging to H-1 of the α-linked Fuc3NAc residue and three broadened singlets to H-1 of the rhamnose residues.

Pre-irradiation of H-1 of one of the rhamnose residues (unit **A**) at 5.10 ppm resulted in a considerable NOE response on H-2 of another rhamnose residue (unit **B**) at 4.22 ppm. In turn, in the spectrum obtained with pre-irradiation of H-1 of unit **B** at 5.19 ppm, there were observed an NOE on H-3 of the third rhamnose residue (unit **C**) at 3.89 ppm, and pre-irradiation of H-1 of unit **C** at 5.01 ppm caused an NOE on H-3 of unit **A** at 3.83 ppm (Fig. 2). These data showed that three rhamnose residues form the main chain of the polysaccharide, where units **A** and **C** are substituted at position 3 and unit **B** at position 2.

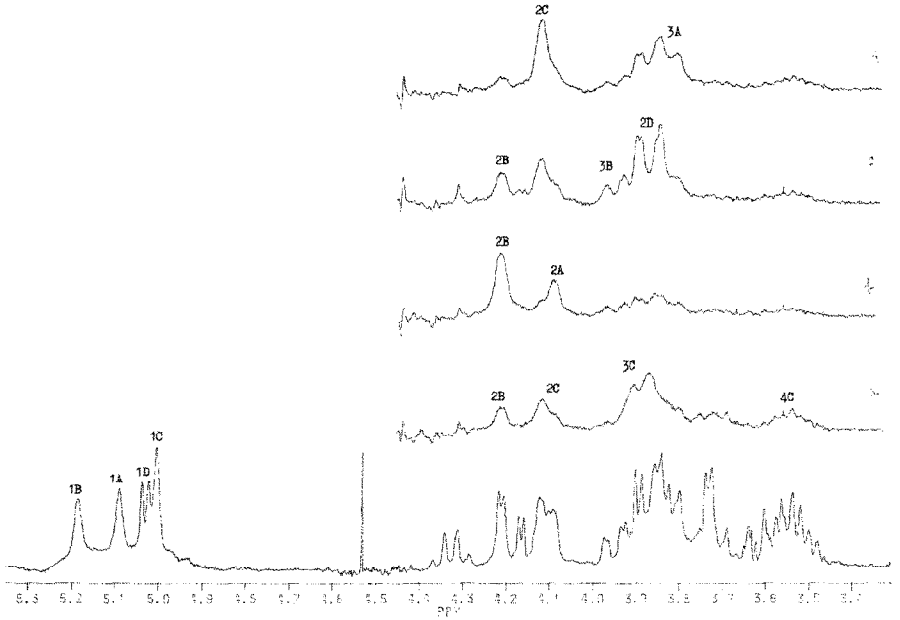

Fig. 2. Part of a 250-MHz ^{1}H NMR spectrum of the O-specific polysaccharide (bottom curve) and NOE spectra with pre-irradiation of H-1 of units **B**, **A**, **D**, and **C** (curves a, b, c, and d, respectively).

Besides the above-mentioned responses, in the NOE spectra, there were observed considerable responses on H-2 (but not H-3 and H-5) of each of the pre-irradiated sugar residues, which confirmed the α configuration of the rhamnosyl linkages. Only small NOEs, which were caused by spin diffusion characteristic of polymers, arose on H-2 of units **A** and **C** as a result of pre-irradiation of H-1 of units **C** and **B**, respectively. This was additional evidence of the same (L) absolute configuration of the three rhamnose residues since, in the α-(1 → 3)-linked disaccharide of rhamnopyranoses with different absolute configurations, the NOE on H-2 is known[6] to be twice as intense as that on H-3.

Furthermore, pre-irradiation of H-1 of the FucNAc residue (unit **D**) caused considerable NOEs on H-2 and H-3 of unit **B**, proving the attachment of unit **D** as a side chain to unit **B** at position 3. The appearance of the NOE on both H-2 and H-3 is typical[6] of disaccharide units with L-rhamnopyranose, 3-substituted by an α-pyranoside having the opposite absolute configuration (i.e., the D configuration).

Assignment of the ^{13}C NMR spectrum of the polysaccharide, by using 2D heteronuclear ^{13}C–^{1}H shift-correlated spectroscopy (Fig. 1, Table I), was in agreement with the results of the NOE linkage analysis. In particular, the relatively low-field positions of the signals for C-3 of units **A** and **C** at 78.9 and 79.2 ppm, respectively, and for C-2 and C-3 of unit **B** at 76.2 and 77.5 ppm, respectively, as compared to their positions in the spectra of the corresponding free monosaccharides, were due to the α-effects of glycosylation[7] and confirmed independently the substitution patterns of the sugar residues established by using NOE spectroscopy

(see above). The high-field position of the signal for C-1 of the Fuc3NAc residue at 97.5 ppm, which corresponded to a small glycosylation effect (ca. 4 ppm), confirmed[8,9] the different absolute configurations of Fuc3NAc and rhamnose, confirming the D configuration of Fuc3NAc (a chemical shift of ~ 101 ppm would be expected if Fuc3NAc and rhamnose had the same absolute configuration).

The data obtained showed that the O-specific polysaccharide of *P. syringae* pv. *tomato* 140(R) has the structure **1**.

$$\begin{array}{l} \qquad\qquad\qquad\qquad\quad \alpha\text{-D-Fuc}p3\text{NAc} \quad \mathbf{D} \\ \qquad\qquad\qquad\qquad\qquad\quad 1 \\ \qquad\qquad\qquad\qquad\qquad\quad \downarrow \\ \qquad\qquad\qquad\qquad\qquad\quad 3 \\ \rightarrow 3)\text{-}\alpha\text{-L-Rha}p\text{-}(1 \rightarrow 2)\text{-}\alpha\text{-L-Rha}p\text{-}(1 \rightarrow 3)\text{-}\alpha\text{-L-Rha}p\text{-}(1 \rightarrow \\ \qquad\quad \mathbf{A} \qquad\qquad\qquad \mathbf{B} \qquad\qquad\qquad \mathbf{C} \end{array}$$

1 [*Pseudomonas syringae* pv. *tomato* 140(R)]

$$\begin{array}{l} \qquad\qquad\qquad\qquad\qquad\qquad\quad \alpha\text{-D-Fuc}p3\text{NAc} \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad 1 \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \downarrow \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad 2 \\ \rightarrow 3)\text{-}\alpha\text{-L-Rha}p\text{-}(1 \rightarrow 2)\text{-}\alpha\text{-L-Rha}p\text{-}(1 \rightarrow 3)\text{-}\alpha\text{-L-Rha}p\text{-}(1 \rightarrow \end{array}$$

2 (*Pseudomonas syringae* pv. *tabaci* 223)

The O-antigen of pv. *tomato* clearly belongs to the structural type characteristic of O-antigens of other *P. syringae* pathovars studied previously (refs. 1–3 and refs. therein), which consist of a linear rhamnan or have a rhamnan backbone with a monosaccharide side chain. It is most closely related to the O-antigen of pv. *tabaci* (structure **2**), which has the same backbone and lateral Fuc3NAc residue but differs in the site of attachment of the side chain[10]. Such a similarity of the O-antigens substantiates serological cross-reactivity of these two strains, which served as a basis of their placing[11] in the same serogroup VII.

O-antigens of *P. syringae* pathovars belonging to serogroup I possess the same Fuc3NAc side chain but differ in the structure of the rhamnan main chain, which lacks strict regularity, being built up of tetrasaccharide units of two types[9,12]. The O-antigens of various pathovars belonging to serogroup I differ from each other in the same manner as those belonging to serogroup VII. It is noteworthy that the backbone of the O-antigens of pvs. *tomato* and *tabaci* (structures **1** and **2**) is identical to that of some pathovars of serogroup II (refs. 1,13,14), except that it contains L-rhamnose instead of D-rhamnose.

EXPERIMENTAL

Optical rotation was measured with a Jasco DIP 360 polarimeter for a solution in water at 25°C.

^{1}H NMR spectra were recorded with a Bruker WM-250 instrument for solutions in D_2O at 55°C. ^{13}C NMR spectra were recorded with a Bruker AM-300 instrument for solutions in D_2O at 60°C. Acetone was used as an internal standard (δ_H 2.23 ppm, δ_C 31.45 ppm). Selective spin decoupling, 1D NOE, and 2D homonuclear ^{1}H–^{1}H and heteronuclear ^{13}C–^{1}H shift-correlated spectroscopy (COSY) were carried out as described[15,16].

Growth of bacteria, isolation of lipopolysaccharide, and isolation of O-specific polysaccharide were performed as described[4,17].

For sugar analysis, the polysaccharide was hydrolysed with 2 M trifluoroacetic acid (100°C, 2 h), and the hydrolysate was analysed by using GLC of alditol acetates and an amino acid analyzer[9].

REFERENCES

1 E.V. Vinogradov, A.S. Shashkov, Y.A. Knirel, G.M. Zdorovenko, L.P. Solyanik, and R.I. Gvozdyak, *Carbohydr. Res.*, 212 (1991) 295–299.
2 A.S. Shashkov, E.V. Vinogradov, E.D. Daeva, Y.A. Knirel, G.M. Zdorovenko, N.Y. Gubanova, L.M. Yakovleva, and I.Y. Zakharova, *Carbohydr. Res.*, 212 (1991) 301–305.
3 E.V. Vinogradov, A.S. Shashkov, Y.A. Knirel, G.M. Zdorovenko, L.P. Solyanik, N.Y. Gubanova, and L.M. Yakovleva, *Carbohydr. Res.*, 212 (1991) 307–311.
4 G.M. Zdorovenko, L.M. Yakovleva, R.I. Gvozdyak, I.Y. Zakharova, and L.N. Koshechkina, *Mikrobiol. Zh.*, 44 (1982) 65–70.
5 K. Leontein, B. Lindberg, and J. Lönngren, *Carbohydr. Res.*, 62 (1978) 359–362.
6 G.M. Lipkind, A.S. Shashkov, S.S. Mamyan, and N.K. Kochetkov, *Carbohydr. Res.*, 181 (1989) 1–12.
7 G.M. Lipkind, A.S. Shashkov, Y.A. Knirel, E.V. Vinogradov, and N.K. Kochetkov, *Carbohydr. Res.*, 175 (1989) 59–75.
8 A.S. Shashkov, G.M. Lipkind, Y.A. Knirel, and N.K. Kochetkov, *Magn. Reson. Chem.*, 26 (1988) 735–747.
9 Y.A. Knirel, G.M. Zdorovenko, A.S. Shashkov, L.M. Yakovleva, N.Y. Gubanova, and R.I. Gvozdyak, *Bioorg. Khim.*, 14 (1988) 172–179.
10 A.S. Shashkov, G.M. Zdorovenko, E.D. Daeva, L.M. Yakovleva, L.P. Solyanik, R.I. Gvozdyak, Y.A. Knirel, and N.K. Kochetkov, *Bioorg. Khim.*, 16 (1990) 90–97.
11 L.T. Pastushenko and I.D. Simonovich, *Mikrobiol. Zh.*, 41 (1979) 330–339.
12 Y.A. Knirel, G.M. Zdorovenko, A.S. Shashkov, S.S. Mamyan, N.Y. Gubanova, L.M. Yakovleva, and L.P. Solyanik, *Bioorg. Khim.*, 14 (1988) 180–186.
13 Y.A. Knirel, G.M. Zdorovenko, L.M. Yakovleva, A.S. Shashkov, L.P. Solyanik, and I.Y. Zakharova, *Bioorg. Khim.*, 14 (1988) 166–171.
14 A.R.W. Smith, S.E. Zamze, S.M. Munro, K.J. Carter, and R.C. Hignet, *Eur. J. Biochem.*, 149 (1985) 73–78.
15 N.A. Kocharova, K. Hatano, A.S. Shashkov, Y.A. Knirel, N.K. Kochetkov, and G. Pier, *J. Biol. Chem.*, 264 (1989) 15569–15573.
16 E.V. Vinogradov, Z. Sidorczyk, A. Swierzko, A. Rozalski, E.D. Daeva, A.S. Shashkov, Y.A. Knirel, and N.K. Kochetkov, *Eur. J. Biochem.*, 197 (1991) 93–103.
17 Y.A. Knirel, G.M. Zdorovenko, V.M. Dashunin, L.M. Yakovleva, A.S. Shashkov, I.Y. Zakharova, R.I. Gvozdyak, and N.K. Kochetkov, *Bioorg. Khim.*, 12 (1986) 1253–1262.