Carbohydrate Research, 212 (1991) 295–299
Elsevier Science Publishers B.V., Amsterdam

Note

Somatic antigens of pseudomonads: structure of the O-specific polysaccharide chain of *Pseudomonas syringae* pv. *syringae (cerasi)* 435 lipopolysaccharide

Eugeny V. Vinogradov, Alexander S. Shashkov, Yuriy A. Knirel*,
N. D. Zelinsky Institute of Organic Chemistry, Academy of Sciences of the U.S.S.R., Moscow (U.S.S.R.)

Galina M. Zdorovenko, Lyudmila P. Solyanik, and Rostislav I. Gvozdyak
D. K. Zabolotny Institute of Microbiology and Virology, Academy of Sciences of the Ukrainian S.S.R., Kiev (U.S.S.R.)

(Received July 6th, 1990; accepted for publication, September 25th, 1990)

In studies of the somatic antigens of phytopathogenic pseudomonads, the structures have been established[1–7] of the O-specific polysaccharide chains of various pathovars of *Pseudomonas syringae* and closely related species [*P. syringae* pv. *atrofaciens*, *P syringae* pv. *syringae (cerasi)*, *P. syringae* pv. *syringae (holci)*, *P. wieringae*, and others] belonging to serogroups I–IV, VI, and VII]. We now report the structure of the O-antigen of *P. syringae* pv. *syringae (cerasi)* strain 435 which, serologically, is similar to *P. syringae* pv. *syringae (cerasi)* strain 467 studied earlier[2]. Both of these strains are classified[8] as belonging to the serogroup II, but they differ in the structure of the O-chain of the lipopolysaccharide.

As with lipopolysaccharides of other strains of *P. syringae* and related species,

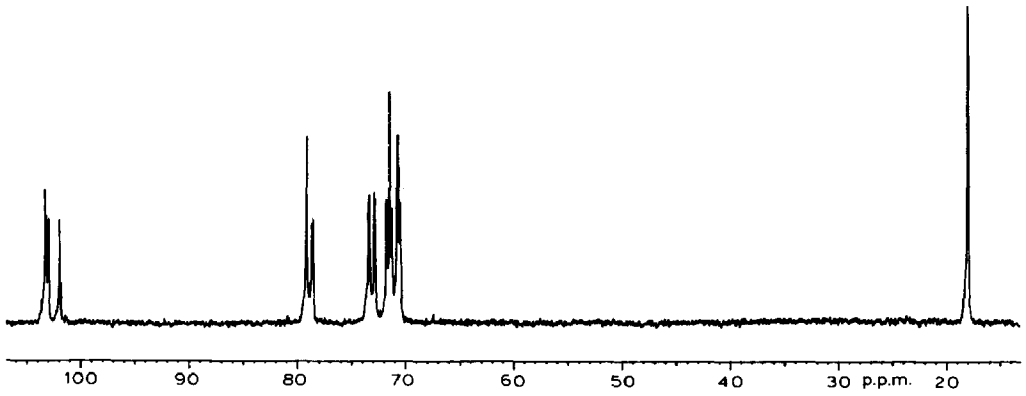


Fig. 1. ^{13}C-N.m.r. spectrum of the O-specific polysaccharide.

*Author for correspondence.

0008-6215/91/$ 03.50 © 1991 – Elsevier Science Publishers B.V.

that of *P. syringae* pv. *syringae (cerasi)* 435 was loosely bound to the cell surface and was isolated by washing the bacterial cells with saline[9]. The lipopolysaccharide was cleaved with dilute acetic acid, and the O-specific polysaccharide, isolated by gel-permeation chromatography, had $[\alpha]_D$ +87° (*c* 1.8, water).

G.l.c. of the derived alditol acetates indicated that the polysaccharide contained only rhamnose, which g.l.c. of the (+)- and (−)-2-octyl glycosides[10] indicated to be D.

The ^{13}C-n.m.r. spectrum (Fig. 1) showed the polysaccharide to comprise a tetrasaccharide repeating-unit (the spectrum contained signals for anomeric carbons at 101.9, 103.0, 103.2, and 103.3 p.p.m.). The position of signals for C-6 at 18.0 p.p.m. and the absence of signals from the region of 82–88 p.p.m. proved that all the residues were pyranosidic.

Methylation analysis of the polysaccharide gave 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylrhamnitol, 1,3,5-tri-*O*-acetyl-2,4-di-*O*-methylrhamnitol, and 1,2,3,5-tetra-*O*-acetyl-4-*O*-methylrhamnitol in the ratios ~1:2:1, which corresponded to the presence in the repeating unit of one lateral, two 3-substituted, and one 2,3-disubstituted rhamnopyranose residue, and indicated the polysaccharide to be branched.

Smith degradation of the polysaccharide removed the lateral rhamnose residue and gave a linear polysaccharide which had a ^{13}C-n.m.r. spectrum identical to that of the common polysaccharide antigen of *Pseudomonas aeruginosa*[11] and the O-specific polysaccharide of *P. syringae* pv. *morsprunorum* C28 (ref. 12) and *P. syringae* pv. *syringae (cerasi)* 467 (ref. 2) with the structure **1**.

→3)-α-D-Rha*p*-(1→3)-α-D-Rha*p*-(1→2)-α-D-Rha*p*-(1→
A B C

1

The results of methylation analysis indicated that the original polymer contained a 2,3-disubstituted rhamnose residue instead of the 2-substituted residue (unit C) in **1** and, therefore, that the lateral rhamnosyl group (unit D) was attached to unit C at O-3. Like units A–C, unit D was α, which was indicated by the ^{13}C-n.m.r. data for the original polysaccharide, interpreted using the data for **1** (ref. 2) and the trisaccharide glycoside **2** (ref. 13) (Table I). In particular, the position of the signals for C-5 at 70.5–70.7 p.p.m. proved that all the rhamnose residues have the α configuration[14], and, therefore, that the O-specific polysaccharide has structure **3**.

D
α-L-Rha*p*
1
↓
3
α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→OMe
B C

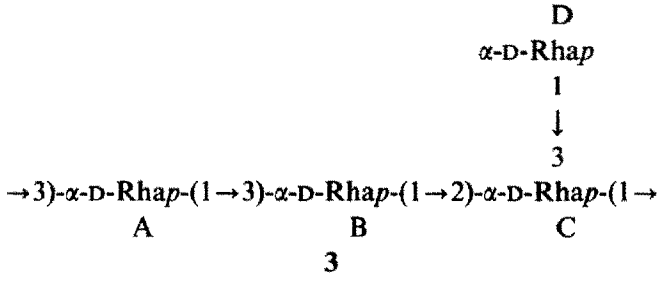
2

TABLE I

^{13}C-N.m.r. chemical shifts[a] (δ in p.p.m.)

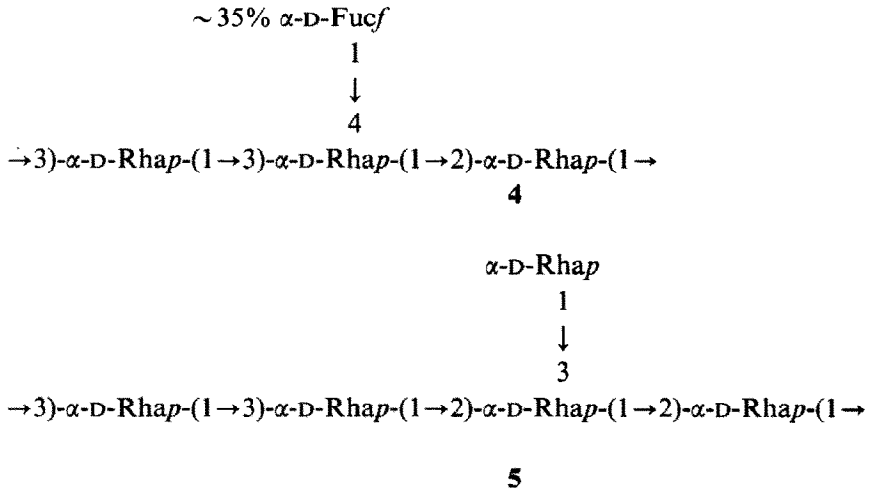
Unit	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
Smith-degraded polysaccharide **1**						
→3)-α-D-Rha*p*-(1→ (A)	103.4	71.1	79.4	72.8	70.6	17.9
→3)-α-D-Rha*p*-(1→ (B)	103.2	71.1	79.3	72.5	70.4	17.9
→2)-α-D-Rha*p*-(1→ (C)	102.0	79.0	71.1	73.4	70.4	17.9
Trisaccharide **2** (*ref. 13*)						
→2)-α-L-Rha*p*-(1→ (C)	100.9	78.8	78.8	73.3	70.2	18.1
3 ↑						
α-L-Rha*p*-(1→ (D)	103.6	71.5	71.6	73.3	70.6	18.0
O-*Specific polysaccharide* **3**						
→3)-α-D-Rha*p*-(1→ (A)	103.3	71.5	79.1	72.9	70.7	18.0
→3)-α-D-Rha*p*-(1→ (B)	103.2	71.5	79.1	72.8	70.6	18.0
→2)-α-D-Rha*p*-(1→ (C)	101.9	78.5	78.6	73.3	70.6	18.0
3 ↑						
α-D-Rha*p*-(1→ (D)	103.0	71.7	71.3	73.3	70.5	18.0

[a] Assignments of signals having differences in chemical shifts of <0.5 p.p.m. could be interchanged.



3

The structure **3** of the *P. syringae* pv. *syringae (cerasi)* 435 O-antigen is related, but not identical, to those of other phytopathogenic pseudomonads that belong to the serogroup II. Thus, the main chain of the polysaccharide **3** has the same tructure (**1**) as the O-antigen of another representative of *P. syringae* pv. *syringae (cerasi)* strain 467 (ref. 2) and *P. syringae* pv. *morsprunorum* C28 (ref. 12), as well as the main chain of *P. syringae* pv. *syringae* 218 in which ~35% of the repeating units (**4**) are substituted by a lateral α-D-fucofuranosyl group[2]. The O-antigens of *P. syringae* pv. *syringae (holci)* K-1025 and *P. syringae* pv. *atrofaciens* 90a differ from that of *P. syranges* pv. *syringae (cerase)* 435 in the structure of the main chain, which is a D-rhamnan with a tetrasaccharide repeating-unit (**5**) but have the same (1→3)-linked lateral α-D-rhamnosyl substituent[3].



4

5

Therefore, as judged from the structures of the O-specific polysaccharides, serogroup II in the classification of phytopathogenic pseudomonads[8] is heterogeneous and should be divided into subgroups.

EXPERIMENTAL

General methods. — ^{13}C-N.m.r. spectra were recorded with a Bruker AM-300 instrument for solutions in D_2O at 60° (internal acetone, δ 31.45). Optical rotations were measured with a Jasco DIP 300 polarimeter at 25°.

G.l.c. was performed with a Hewlett–Packard 5890 instrument equipped with a flame-ionisation detector and a glass capillary column (0.2 mm × 25 m) coated with OV-1. G.l.c.–m.s. was carried out with a Varian MAT 311 instrument under the same conditions as in g.l.c. Gel-permeation chromatography was performed (*a*) on a column (3.5 × 70 cm) of Sephadex G-50 in a pyridine acetate buffer (pH 5.5), with the fractions analysed by the phenol–sulfuric acid method[15]; or (*b*) on a column (80 × 1.6 cm) of TSK HW 40 (S) in water, with the elution monitored by a Knauer differential refractometer. Neutral sugars were analysed on a column (0.6 × 20 cm) of Durrum DAx4 resin in 0.5M sodium borate buffer (pH 9.0) at 65°, and the elution was monitored by the orcinol–sulfuric acid reaction, using a Technicon Autoanalyser II.

The growth of bacteria and the isolation of the lipoplysaccharide and the O-specific polysaccharide were carried out as described[1,9].

Samples were hydrolysed with 2M trifluoroacitic acid in sealed ampoules for 2 h at 120°. Methylation analysis was performed according to the Hakomori procedure[16] and the products were recovered using a Sep-Pak cartridge[17].

Smith degradation. — The O-specific polysaccharide (18 mg) was treated with 0.1M sodium metaperiodate (2 mL) for 24 h at room temperature in the dark, the product was reduced with an excess of sodium borohydride, desalted by gel-permeation chromatography on TSK HW 40, and hydrolysed with aqueous 2% acetic acid (100°, 2

h), and the modified polysaccharide (10 mg) was isolated by gel-permeation chromatography on TSK HW 40.

REFERENCES

1 Y. A. Knirel, G. M. Zdorovenko, V. M. Dashunin, L. M. Yakovleva, A. S. Shashkov, I. Y. Zakharova, R. I. Gvozdiak, and N. K. Kochetkov, *Bioorg. Khim.*, 12 (1986) 1253–1262.
2 Y. A. Knirel, G. M. Zdorovenko, A. S. Shashkov, S. S. Mamyan, L. M. Yakovleva, L. P. Solyanik, and I. Y. Zakharova, *Bioorg. Khim.*, 14 (1988) 82–91.
3 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.
4 Y. A. Knirel, G. M. Zdorovenko, A. S. Shashkov, N. I. Gubanova, L. M. Yakovleva, and R. I. Gvozdiak, *Bioorg. Khim.*, 14 (1988) 92–99.
5 Y. A. Knirel, G. M. Zdorovenko, A. S. Shashkov, L. M. Yakovleva, N. I. Gubanova, and R. I. Gvozdiak, *Bioorg. Khim.*, 14 (1988) 172–179.
6 Y. A. Knirel, G. M. Zdorovenko, A. S. Shashkov, S. S. Mamyan, N. I. Gubanova, L. M. Yakovleva, and L. P. Solyanic, *Bioorg. Khim.*, 14 (1988) 180–186.
7 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.
8 L. T. Pastushenko and I. D. Simonovich, *Mikrobiol. Zh.*, 41 (1979) 330–339.
9 G. M. Zdorovenko, L. M. Yakovleva, R. I. Gvozdiak, I. Y. Zakharova, and L. N. Koshechkina, *Mikrobiol. Zh.*, 44 (1982) 65–70.
10 K. Leontein, B. Lindberg, and J. Lönngren, *Carbohydr. Res.*, 62 (1978) 359–362.
11 N. A. Kocharova, Y. A. Knirel, N. K. Kochetkov, and E. S. Stanislavsky, *Bioorg. Khim.*, 14 (1988) 701–703.
12 A. R. Smith, S. E. Zamze, S. M. Munro, K. J. Carter, and R. C. Hignett, *Eur. J. Biochem.*, 141 (1985) 73–78.
13 G. M. Lipkind, N. E. Nifant'ev, A. S. Shashkov, and N. K. Kochetkov, *Can. J. Chem.*, 68 (1990) 1238–1250.
14 K. Bock and C. Pedersen, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 27–65.
15 M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1965) 350–356.
16 S. Hakomori, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
17 T. J. Waeghe, A. G. Darvill, M. McNeil, and P. Albersheim, *Carbohydr. Res.*, 123 (1983) 281–304.