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Note

Somatic antigens of pseudomonads: structure of the O-specific polysaccharide chain of *Pseudomonas syringae* pv. *tabaci* 225 (serogroup VIII) lipopolysaccharide

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In a study[1] of the lipopolysaccharides of *Pseudomonas syringae* and related species, the structure of the O-antigen of *P. syringae* pv. *tabaci* strain 223 belonging to serogroup VII has been established. We report now the structure of the O-specific polysaccharide of another representative of this pathovar, namely, *P. syringae* pv. *tabaci* strain 225, which belongs to serogroup VIII.

As for the loosely bound lipopolysaccharides of many other phytopathogenic pseudomonads[1–4], that of strain 225 was isolated from bacterial cells by extraction with saline[5]. The lipopolysaccharide was cleaved with dilute acetic acid and the O-specific polysaccharide was isolated by gel-permeation chromatography on Sephadex G-50.

The ^{13}C-n.m.r. spectrum of the polysaccharide (Fig. 1) contained signals for four anomeric carbons at 97.0, 97.2, 101.8, and 102.0 p.p.m., three methyl groups (C-6) of 6-deoxy sugars at 17.8–18.1 p.p.m., one hydroxymethyl methyl group (C-6) at 63.0 p.p.m., one carbon bearing nitrogen at 57.2 p.p.m., 15 other sugar ring carbons in the region 67.8–84.0 p.p.m., and one *N*-acetyl group (CH_3 at 23.3 p.p.m., CO at 175.5 p.p.m.).

G.l.c. of alditol acetates derived from the polysaccharide revealed rhamnose and 2-amino-2-deoxyglucose in the ratio ~3:1. Determination[6] of the absolute configuration of rhamnose revealed the D and L forms in the ratio ~1:2. 2-Amino-2-deoxyglucose, isolated from the hydrolysate of the polysaccharide by preparative p.c., had a positive $[\alpha]_D$ which indicated the D configuration.

These data indicated that the polysaccharide had a tetrasaccharide repeating-unit that contained residues of D-rhamnose, L-rhamnose (2), and 2-acetamido-2-deoxy-D-glucose.

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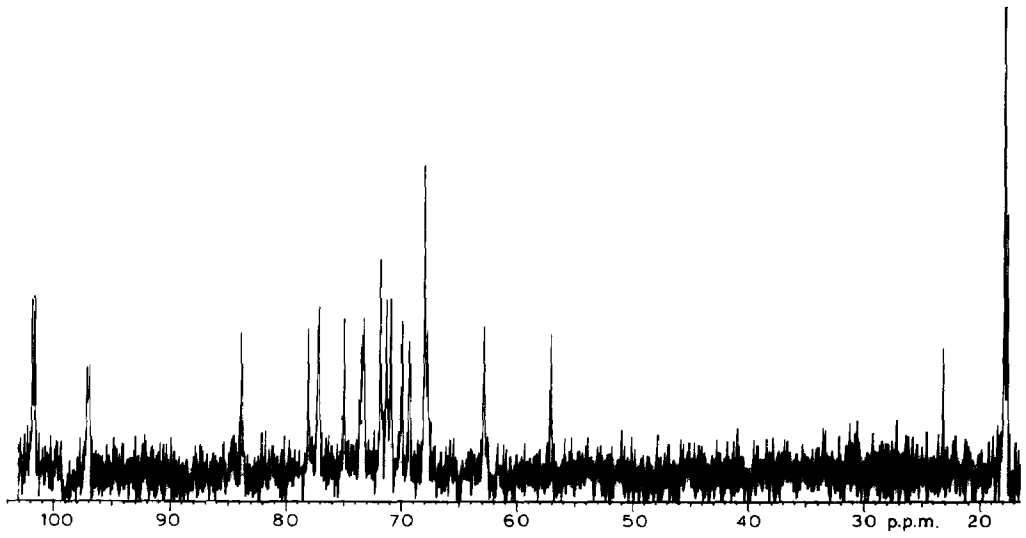


Fig. 1. ^{13}C-N.m.r. spectrum of the O-specific polysaccharide (except for the signal for the CO group).

Methylation analysis of the polysaccharide gave approximately equal amounts of 1,3,5-tri-*O*-acetyl-2,4-di-*O*-methylrhamnitol, 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylrhamnitol, 1,3,4,5-tetra-*O*-acetyl-2-*O*-methylrhamnitol, and 1,5-di-*O*-acetyl-2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)glucitol. Thus, the polysaccharide was branched and contained residues of 3-, 4-, and 3,4-di-substituted rhamnose and a lateral residue of 2-acetamido-2-deoxyglucose.

The polysaccharide was *N*-deacetylated with anhydrous hydrazine in the presence of hydrazine sulphate[7], then deaminated with nitrous acid. The polymeric product, isolated by gel-permeation chromatography on TSK HW 40, had a ^{13}C-n.m.r. spectrum that was identical to that[3] of the O-specific polysaccharide of *P. syringae* pv. *lachrymans* 7591, which had structure **1**.

$$\underset{\mathbf{A}}{\rightarrow 3)\text{-}\beta\text{-L-Rha}p\text{-}(1\rightarrow 4)}\text{-}\alpha\text{-L-}\underset{\mathbf{B}}{\text{Rha}p\text{-}(1\rightarrow 3)}\text{-}\alpha\text{-D-}\underset{\mathbf{C}}{\text{Rha}p\text{-}(1\rightarrow}$$

1

Thus, the main chain of the polysaccharide has the structure **1** and the residue of 2-acetamido-2-deoxyglucose (unit D) is attached at position 4 of one of the 3-substituted rhamnose residues (unit A or C).

In order to decide between these two possibilities, advantage was taken of the stability[7] of 2-amino-2-deoxyglycosidic linkages towards acid hydrolysis. Hydrolysis of the *N*-deacetylated polysaccharide with 2M hydrochloric acid gave the disaccharide GlcN→Rha together with monosaccharides. Deamination with nitrous acid of the amino components, isolated by cation-exchange chromatography, released D-rhamnose; hence, unit D was attached to unit C.

The chemical shift (57.2 p.p.m.) of the signal for C-2 of unit D in the ^{13}C-n.m.r. spectrum of the polysaccharide was characteristic[8] for the residue of 2-acetamido-2-deoxy-β-D-glucose; hence, unit D was β-linked.

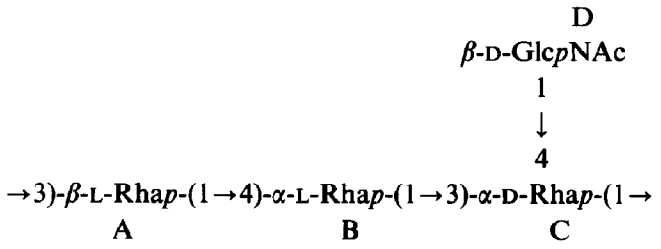
Thus, it was concluded that the O-specific polysaccharide of *P. syringae* pv. *tabaci* strain 225 has the structure **2**, which accords with the ^{13}C-n.m.r. assignments (Table I) made on the basis of a comparison with the spectrum of polysaccharide **1** and with other data[9].

TABLE I

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

Unit	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
N-*Deacetylated-deaminated polysaccharide* **1**						
→3)-β-L-Rha*p*-(1→ (A)	101.7	68.2	78.2	71.6	73.7	17.9
→4)-α-L-Rha*p*-(1→ (B)	97.9	71.4	70.5	84.0	68.6	18.1
→3)-α-D-Rha*p*-(1→ (C)	97.7	68.2	76.6	71.7	70.2	18.2
O-*Specific polysaccharide* **2**[a,b]						
→3)-β-L-Rha*p*-(1→ (A)	101.8	67.8	78.1	71.9	73.5	17.8
→4)-α-L-Rha*p*-(1→ (B)	97.2	71.4	70.1	84.0	68.1	18.1
→3)-α-D-Rha*p*-(1→ (C)	97.0	68.1	73.4	77.2	69.4	18.1
4 ↑						
β-D-Glc*p*NAc-(1→ (D)	102.0	57.2	75.1	71.0	77.3	63.0

[a] Assignments of signals having differences in chemical shifts of < 0.5 p.p.m. could be interchanged. [b] NAc at 23.3 (CH_3) and 175.5 (CO) p.p.m.

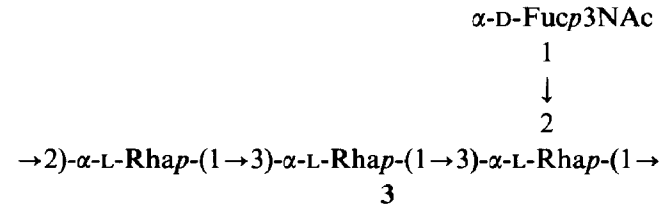


2

The O-antigen of *P. syringae* pv. *tabaci* 225 (serogroup VIII) is related structurally to that[3] of *P. syringae* pv. *lachrymans* 7591 (serogroup IX). These O-antigens have the same rhamnan backbone, but differ by the attachment of a lateral residue of 2-acetamido-2-deoxy-D-glucose in the former polysaccharide (structures **2** and **1**, respectively). The presence of this amino sugar in the O-antigen of serogroup VIII may be responsible for the absence of any serological cross-reaction between strains of serogroups VIII and IX.

The strains of these two serogroups differ from the strains of other serogroups of *P. syringae* and related species in the structure of the O-antigen. Thus, their O-antigens contain both D- and L-rhamnose and a β-rhamnose residue, whereas those of the strains that belong to the serogroups I–VII are α-L- or α-D-rhamnans (ref. 2 and papers cited therein). This difference correlates well with subdivision of these strains on the basis of DNA–DNA hybridisation data[10], and, hence, in the *P. syringae* group, the chemical features of O-antigens can serve as taxonomic criteria.

The O-antigen[1] of strain 223 of *P. syringae* pv. *tabaci* (serogroup VII) differs significantly in structure from that of strain 225 of the same pathovar (serogroup VIII). Thus, the former has an α-L-rhamnan main chain and a lateral residue of 3-acetamido-3,6-dideoxy-D-galactose (structure **3**). This difference in structure accords with the serological heterogeneity of strains that belong to the pathovar *tabaci* and confirms the absence of any correlation between the differentiation of *P. syringae* strains into pathovars (*i.e.*, host specifity) and the structure of the O-antigen.



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 for solutions in water 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 chromatographic 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 monitoring by the phenol–sulfuric acid reaction, or (*b*) on a column (80 × 1.6 cm) of TSK HW 40 (S) in water with monitoring by a Knauer differential refractometer. P.c. was performed on FN-11 paper, using pyridine–ethyl acetate–acetic acid–water (5:5:1:3).

The growth of bacteria and the isolation of the lipopolysaccharide and the O-specific polysaccharide were performed as described[4,5].

Samples were hydrolysed with 2M trifluoroacetic acid in sealed ampoules at 120° for 2 h. Methylation analysis was performed according to the Hakomori procedure[11] and the products were recovered using a Sep-Pak cartridge[12].

N-Deacetylation. — The polysaccharide (35 mg) was dried over phosphorus pentaoxide *in vacuo* and dissolved in anhydrous hydrazine (1 mL) that contained

hydrazine sulphate (50 mg). The solution was heated in a sealed tube for 20 h at 105°, then concentrated, and the resulting *N*-deacetylated polysaccharide was desalted by gel-permeation chromatography on TSK HW 40.

Deamination. — The *N*-deacetylated polysaccharide (20 mg) was dissolved in water (1 mL), aqueous 5% sodium nitrite (1.5 mL) and aqueous 33% acetic acid (1.5 mL) were added, the mixture was kept for 1 h at 20°, and the deaminated polysaccharide was isolated by gel-permeation chromatography on TSK HW 40.

Acid hydrolysis. — The *N*-deacetylated polysaccharide (4 mg) was hydrolysed with 2M hydrochloric acid (100°, 2 h). The hydrolysate was concentrated, a solution of the residue in water was treated with KU-2 (H^+) resin, the resin was washed with water, and the amino components were eluted with M hydrochloric acid. The eluate was concentrated, and to a solution of the residue in water (1 mL) were added aqueous 5% sodium nitrite (1.5 mL) and aqueous 33% acetic acid (1.5 mL). The mixture was kept for 1 h at 20°, treated with KU-2 (H^+) resin, and concentrated at room temperature to give 2,5-anhydromannose[7] and D-rhamnose.

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