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Note

Somatic antigens of pseudomonads: structure of the O-specific polysaccharide chain of *Pseudomonas gladioli* pv. *alliicola* 8494 (serogroup X) lipopolysaccharide

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In continuing the chemical and immunochemical studies[1-3] of the lipopolysaccharides (O-antigens) of phytopathogenic pseudomonads, we now report the structure of the O-specific polysaccharide of *Pseudomonas gladioli* pv. *alliicola* strain 8494 (313), which is classified[4] as belonging to serogroup X in contrast to various pathovars of *Pseudomonas syringae* and related species that belong to serogroups I–IX.

As with the strains of the *P. syringae* group, the lipopolysaccharide of *P. gladioli* pv. *alliicola* was isolated by extraction with saline[5], purified by ultracentrifugation, and cleaved with dilute acetic acid to give the O-specific polysaccharide (PS-I) that was isolated by gel-permeation chromatography on Sephadex G-50.

The lipopolysaccharide was serologically active in the Ouchterlony (two clear lines of precipitation) and passive haemagglutination tests with the homologous O-antiserum (titres 1:10.240-20.480). Both lipopolysaccharide and PS-I inhibited passive haemagglutination in the homologous test-system, the latter being less active (minimal inhibiting doses were 2–4 and 32–64 μg, respectively).

The ^{13}C-n.m.r. spectrum (Fig. 1, Table I) showed that PS-I had a tetrasaccharide repeating-unit (signals for anomeric carbons at 103.3, 100.5, 99.0, and 95.4 p.p.m.) that contained one hexose residue (signal for C-6 at 62.2 p.p.m.), three residues of 6-deoxyhexoses (signals for C-6 at 18.0, 17.9, and 16.4 p.p.m.), and an *O*-acetyl group (signals for CO at 174.7 p.p.m. and CH_3 at 21.4 p.p.m.). The ^{1}H-n.m.r. spectrum of PS-I contained, *inter alia*, signals for methyl groups of three 6-deoxyhexoses (3 d at 1.31, 1.28, and 1.18 p.p.m., $J_{5,6}$ 6 Hz) and one *O*-acetyl group at 2.17 p.p.m. (s).

G.l.c. of the derived alditol acetates and determination[6] of the absolute configurations of the monosaccharides isolated from the hydrolysate by anion-exchange chroma-

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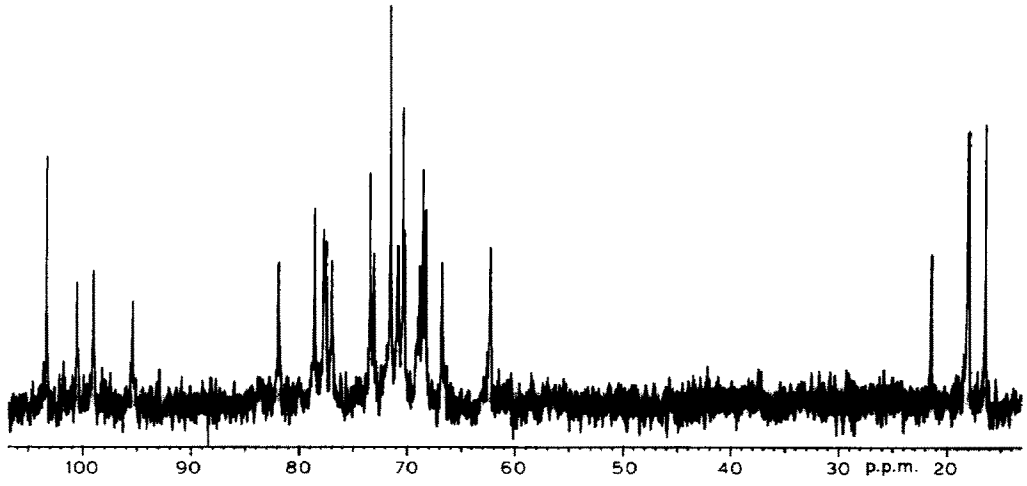


Fig. 1. ^{13}C-N.m.r. spectrum of PS-I (except for the signal for the CO group).

TABLE I

Chemical shifts[a] in the ^{13}C-n.m.r. spectra (δ in p.p.m.)

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
Unit A (α-L-rhamnopyranose)						
PS-I[b]	95.4	77.7	70.8	81.8	68.8	17.9
PS-II	95.1	77.6	71.1	81.4	69.2	17.9
PS-III	95.1	77.4	70.9	81.1	69.2	18.0
PS-IV	97.4	71.8	71.5	80.9	68.7	18.1
	(97.6	71.6	71.3	80.8	68.7	18.0)[c]
Unit B (β-D-mannopyranose)						
PS-I[b]	100.5	70.3	76.9	66.7	77.5	62.2
PS-II	101.9	68.3	78.7	66.4	78.0	62.4
PS-III	101.5	68.3	78.7	66.3	78.1	62.3
PS-IV	101.7	67.9	78.2	66.2	77.4	62.3
	(102.1	68.2	78.5	66.2	77.4	62.3)[c]
Unit C (α-D-fucopyranose)						
PS-I[b]	99.0	68.5	78.5	73.0	68.2	16.4
PS-II	99.2	68.5	78.7	73.1	68.5	16.5
PS-III	99.2	69.0	70.7	73.0	68.1	16.4
Unit D (α-L-rhamnopyranose)						
PS-I[b]	103.3	71.5	71.5	73.4	70.3	18.0
PS-II	103.6	71.5	71.5	73.4	70.4	18.1

[a] Assignments of signals having differences in chemical shifts of <0.5 p.p.m. could be interchanged. [b] OAc at 21.4 p.p.m. (CH_3) and 174.7 p.p.m. (CO). [c] Values calculated by the method[8] are given in parentheses.

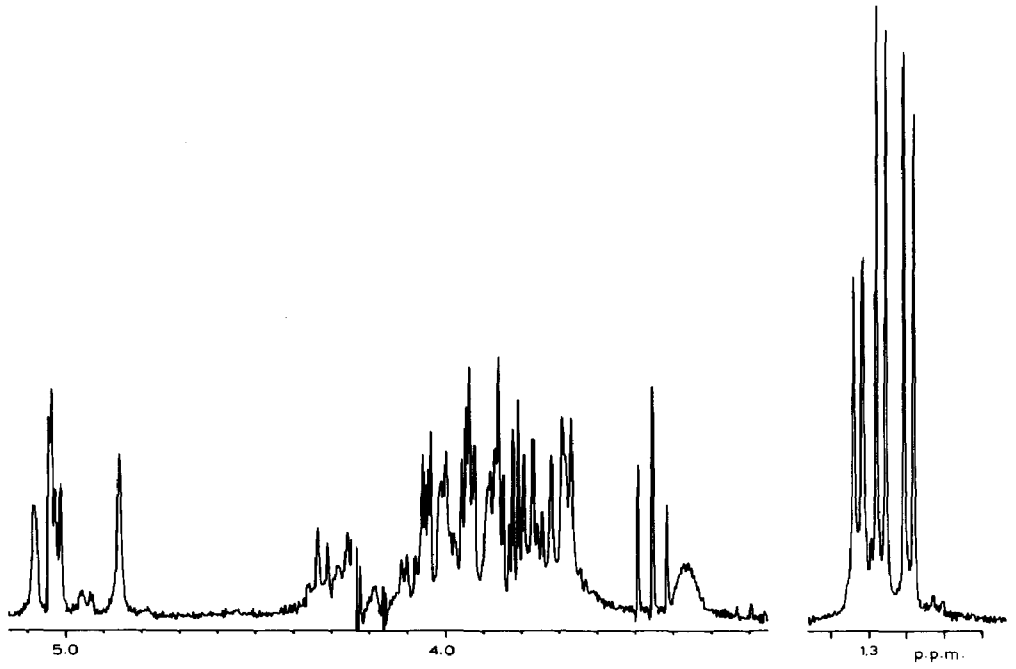


Fig. 2. ^{1}H-n.m.r. spectrum of PS-II (*O*-deacetylated PS-I).

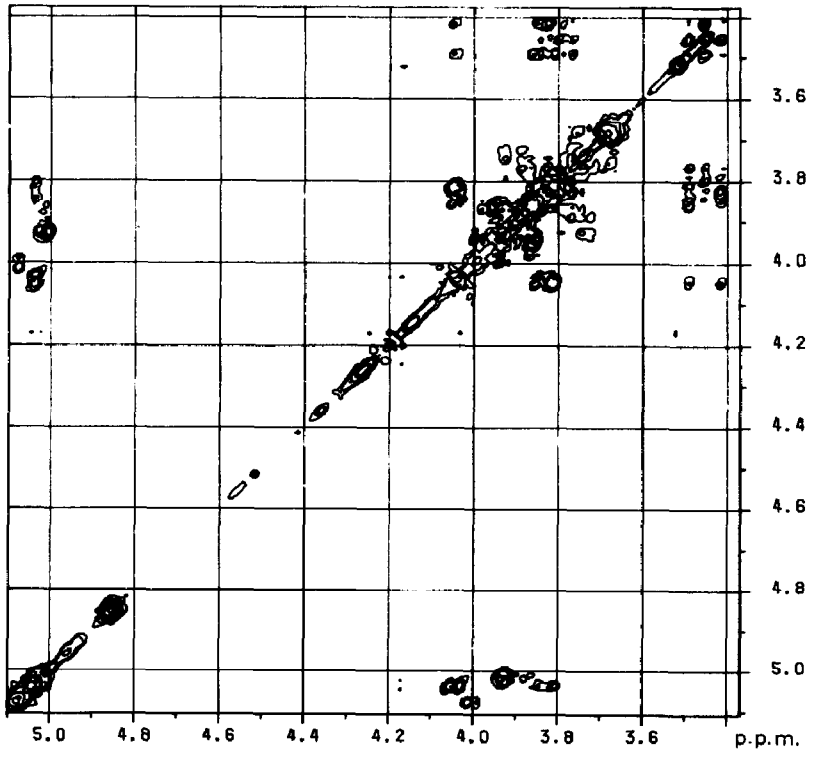


Fig. 3. COSYRCT ^{1}H-n.m.r. spectrum of PS-II.

tography in borate buffer revealed that PS-I contained D-mannose, L-rhamnose, and D-fucose in the ratios ~1:2:1.

PS-I was *O*-deacetylated with aqueous ammonia. The signals in the ^{1}H-n.m.r. spectrum of the product (PS-II) were assigned with the help of sequential, selective spin-decoupling experiments and 2D COSY experiments in combination with single relayed coherence-transfer spectroscopy (COSYRCT) (Figs. 2 and 3, Table II).

The $J_{H,H}$ values (Table II) proved that the sugar residues were pyranosidic and the $J_{1,2}$ value of 3.5 Hz indicated the fucopyranose residue to be α. The anomeric configurations of the other sugar residues and their sequence in the repeating unit were determined by n.O.e. experiments.

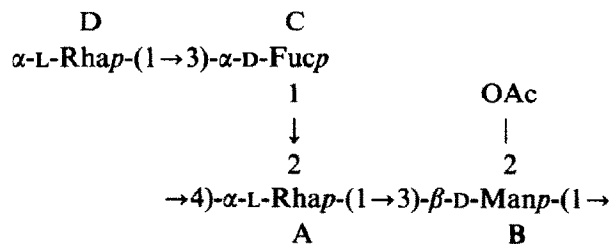
On irradiation of H-1 of the mannopyranose residue (unit B) at 4.86 p.p.m.,

TABLE II

^{1}H-N.m.r. data (δ in p.p.m., J in Hz)

	H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*
PS-II						
Unit A (α-L-rhamnopyranose)						
δ	5.07 (bs)	4.01 (d)	4.08 (dd)	3.73 (t)	4.01 (dq)	1.32 (d)
J	$J_{1,2}$ <1	$J_{2,3}$ 3	$J_{3,4}$ 9	$J_{4,5}$ 9	$J_{5,6}$ 6	
Unit B (β-D-mannopyranose)						
δ	4.86 (bs)	4.26 (d)	3.60–3.74 (m)		3.36 (bs)	3.76, 3.92 (2 dd)
J	$J_{1,2}$ <1	$J_{2,3}$ 2			$J_{5,6}$ 6	$J_{5,6'}$ 5 $J_{6,6'}$ 13
Unit C (α-D-fucopyranose)						
δ	5.01 (d)	3.88 (dd)	3.88 (dd)	3.89 (d)	4.34 (q)	1.18 (d)
J	$J_{1,2}$ 3.5	$J_{2,3}$ 11	$J_{3,4}$ 3		$J_{5,6}$ 6	
Unit D (α-L-rhamnopyranose)						
δ	5.02 (d)	4.04 (dd)	3.84 (dd)	3.44 (t)	3.80 (dq)	1.26 (d)
J	$J_{1,2}$ 1.5	$J_{2,3}$ 3.5	$J_{3,4}$ 9.5	$J_{4,5}$ 9.5	$J_{5,6}$ 6	
PS-I[a]						
Unit A (α-L-rhamnopyranose)						
δ	5.02 (bs)	3.74 (d)	3.93 (dd)	3.71 (t)	3.95 (dq)	1.31 (d)
J	$J_{1,2}$ <1		$J_{3,4}$ 8	$J_{4,5}$ 8	$J_{5,6}$ 6	
Unit B (β-D-mannopyranose)						
δ	5.04 (bs)	5.56 (d)	3.84 (dd)	3.63 (t)	3.44 (m)	
J	$J_{1,2}$ <1	$J_{2,3}$ 4	$J_{3,4}$ 9.5	$J_{4,5}$ 9.5		
Unit C (α-D-fucopyranose)						
δ	4.94 (d)	3.91 (dd)				1.19 (d)
J	$J_{1,2}$ 3.5	$J_{2,3}$ 11			$J_{5,6}$ 6	
Unit D (α-L-rhamnopyranose)						
δ	5.04 (d)	4.06 (dd)	3.85 (dd)	3.46 (t)	3.81 (dq)	1.28 (d)
J	$J_{1,2}$ 1	$J_{2,3}$ 3	$J_{3,4}$ 9.5	$J_{4,5}$ 9.5	$J_{5,6}$ 6	

[a] OAc at 2.17 p.p.m. (s).



4 (PS-I)

The O-antigen of this species (serogroup X) differs significantly in structure from those of the strains of the *P. syringae* group that belong to serogroups I–IX, which are linear rhamnans or branched polysaccharides with a rhamnan backbone[1–3].

EXPERIMENTAL

General methods. — The ^{13}C-n.m.r. spectra were recorded on a Bruker AM-300 instrument for solutions in D_2O at 60° (internal acetone, δ 31.45). The ^1H-n.m.r. and n.O.e. spectra were recorded on a Bruker WM-250 instrument for solutions in D_2O at 30° and 90° (internal acetone, δ 2.23). 2D COSYRCT and XHCORRD spectra and n.O.e. spectra were obtained as described[2].

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, and a temperature gradient 200→290° at 10°/min. G.l.c.–m.s. was performed on a Varian MAT 311 instrument with an ionisation potential of 70 eV and under the same chromatographic conditions as in g.l.c. Optical rotations were measured on a Jasco DIP 300 polarimeter at 25°.

Gel-permeation chromatography was performed on (*a*) 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. 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°. The eluate was monitored by the orcinol–sulfuric acid reaction, using a Technicon Autoanalyser II.

Serological tests, the growth of bacteria, and the isolation of the lipopolysaccharide and the O-specific polysaccharide were performed as described[5,10].

Acid hydrolysis was performed with 2M trifluoroacetic acid in sealed ampoules for 2 h at 120° for g.l.c. analysis of alditol acetates, or with 2M hydrochloric acid for 2 h at 100° for the determination of absolute configurations. Monosaccharides were separated under the conditions of neutral sugar analysis (see above), fractions were analysed with a Technicon Autoanalyser II, and individual sugars were desalted by treatment with KU-2 (H^+) resin and co-evaporation with methanol.

Methylation analysis was performed according to the Hakomori procedure[11] and the products were recovered by using Sep-Pak cartridges[12].

O-Deacetylation. — PS-I (18 mg) was dissolved in water (1 mL), conc. aqueous ammonia (1 mL) was added, and the mixture was kept at room temperature for 16 h, then concentrated to give PS-II, $[\alpha]_D$ $-10°$ (*c* 1, water).

Smith degradation. — PS-II (18 mg) was treated with 0.1M sodium metaperiodate (2 mL) for 24 h at room temperature in the dark, and the product was reduced with sodium borohydride, desalted by gel-permeation chromatography on TSK HW 40, and hydrolysed with aqueous 2% acetic acid (100°, 2 h) to give PS-III, which was isolated by gel-permeation chromatography on TSK HW 40. PS-III (8 mg) was converted into PS-IV in a similar manner.

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