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Note

Somatic antigens of pseudomonads: structure of the O-specific polysaccharide chain of *Pseudomonas syringae* pv. *lachrymans* 7591 (serogroup IX) lipopolysaccharide

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As part of a study of the lipopolysaccharides of phytopathogenic pseudomonads (ref. 1 and papers cited therein), we report now the structure of the O-specific polysaccharide of *Pseudomonas syringae* pv. *lachrymans* strain 7591, which belongs to serogroup IX.

The lipopolysaccharide was isolated from the bacterial cells by extraction with saline[2] and cleaved with dilute acetic acid, and the O-specific polysaccharide, isolated by gel-permeation chromatography on Sephadex G-50, had $[\alpha]_D$ +10° (*c* 3.4, water).

Acid hydrolysis of the polysaccharide gave rhamnose only, which was identified by g.l.c. of the derived alditol acetate. G.l.c. of glycosides of rhamnose with (+)- and (−)-2-octanol[3] indicated the presence of the D and L isomers in the ratio ~1:2.

The ^{13}C-n.m.r. spectrum of the polysaccharide (Fig. 1) contained signals for three anomeric carbons at 97.7, 97.9, and 101.7 p.p.m., three methyl groups (C-6) at 17.9, 18.1, and 18.2 p.p.m., and twelve other sugar ring carbons in the region 68–84 p.p.m.

The ^{1}H-n.m.r. spectrum of the polysaccharide (Fig. 2) contained signals for three anomeric protons at 4.70 (bs), and 4.95 and 4.98 p.p.m. (2 d, $J_{1,2}$ ~1.5 Hz); three methyl groups (H-6,6,6) at 1.26 and 1.27 (2 d, $J_{5,6}$ ~6 Hz), and 1.33 p.p.m. (m); as well as for twelve other sugar protons in the region 3.5–4.3 p.p.m.

These data showed that the polysaccharide has a trisaccharide repeating-unit that contains one D- and two L-rhamnose residues. The position near 18 p.p.m. of signals for C-6 proved that all of the rhamnose residues were pyranosidic.

The ^{1}H-n.m.r. spectrum of the polysaccharide was interpreted by using sequential, selective spin-decoupling experiments (Table I). The relatively high-field positions at 4.70, 3.64, and 3.40–3.51 p.p.m. of the signals for H-1,3,5, respectively, of one of the

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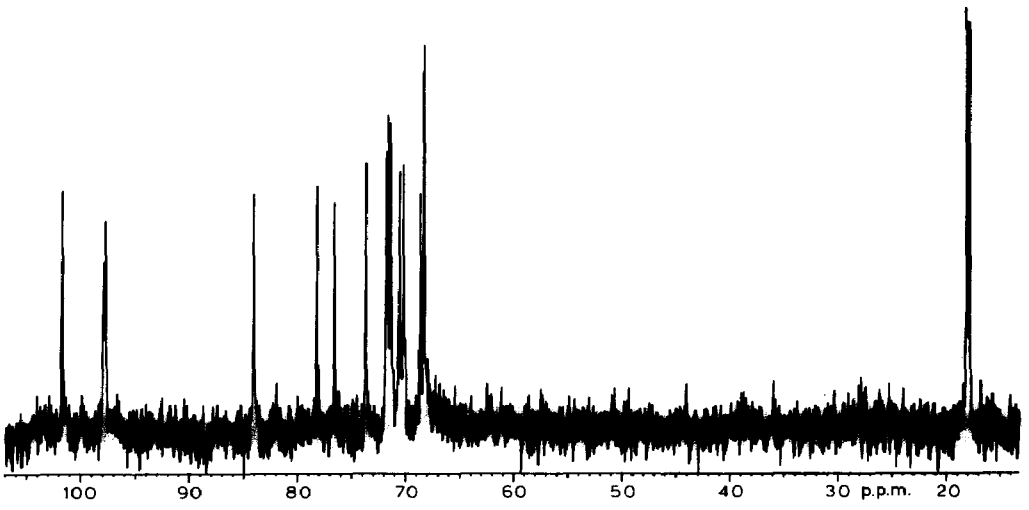

Fig. 1. ^{13}C-n.m.r. spectrum of the O-specific polysaccharide.

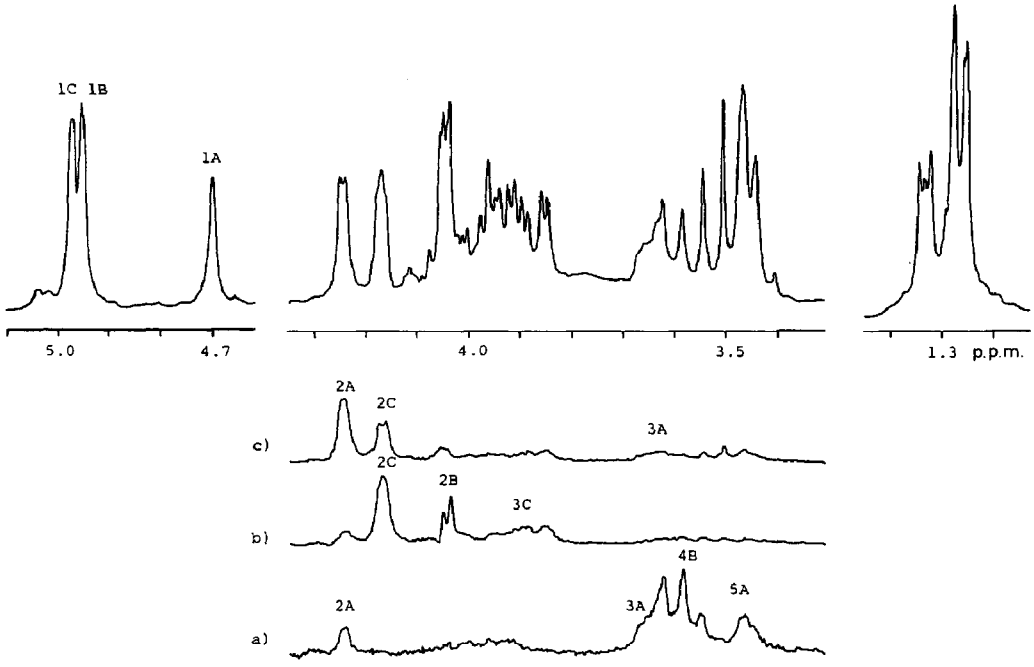

Fig. 2. ^{1}H-N.m.r. spectrum of the O-specific polysaccharide (top curve) and n.O.e. spectra with irradiation of H-1 of units A, B, and C (curves a, b, and c, respectively).

rhamnose residues (unit A) showed that it was β, whereas the other two rhamnose residues (units B and C) with the resonances of H-1,3,5 at lower field (4.95–4.98, 3.87–3.83, and 3.97–4.04 p.p.m., respectively) were α.

Irradiation of H-1 of unit A at 4.70 p.p.m. (Fig. 2a) resulted in n.O.e.'s on H-2,3,5 of this unit, which is consistent with the β configuration, and also on H-4 of unit B at 3.58 p.p.m., which proved units A and B to be joined by a β-(1→4) linkage. N.O.e.'s produced on irradiation of H-1 of units B and C at 4.95 and 4.98 p.p.m. confirmed that these units were α. Determination of positions of attachment of these units was complicated by the facts that the signals for H-1 had similar chemical shifts and the n.O.e. on H-2 may be equal to, or even larger than, that on H-3 in some α-(1→3)-linked rhamnose disaccharides[4]. Nevertheless, the occurrence of n.O.e.'s on H-3 of units C and A at 3.87 and 3.64 p.p.m. as a result of irradiation of H-1 of units B and C (Fig. 2, b and c, respectively) allowed the conclusion that unit B was attached at position 3 of unit C, and unit C at position 3 of unit A. Hence, the polysaccharide is linear.

With the ^{1}H-n.m.r. spectrum assigned, the ^{13}C-n.m.r. spectrum of the polysaccha-

TABLE I

^{1}H-N.m.r. data (δ in p.p.m., J in Hz) for the O-specific polysaccharide

Data	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*
β-L-Rhamnopyranose (unit A)						
δ	4.70 (bs)	4.24 (d)	3.64 (dd)	3.40–3.51 (2 m)		1.33 (d)
J		$J_{2,3}$ 3	$J_{3,4}$ 9		$J_{5,6}$ 6	
α-L-Rhamnopyranose (unit B)						
δ	4.95 (d)	4.04 (dd)	3.93 (dd)	3.58 (t)	4.04 (dd)	1.27 (d)
J	$J_{1,2}$ 1.5	$J_{2,3}$ 3	$J_{3,4}$ 9	$J_{4,5}$ 9	$J_{5,6}$ 6	
α-D-Rhamnopyranose (unit C)						
δ	4.98 (d)	4.17 (dd)	3.87 (dd)	3.50 (t)	3.97 (dd)	1.26 (d)
J	$J_{1,2}$ 1.5	$J_{2,3}$ 3	$J_{3,4}$ 9	$J_{4,5}$ 9	$J_{5,6}$ 6	

TABLE II

^{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*
O-Specific polysaccharide						
→3)-β-L-Rha*p*-(1→ (A)	101.7	68.3	78.2	71.6	73.7	17.9[a]
→4)-α-L-Rha*p*-(1→ (B)	97.9	71.4	70.5	84.0	68.6	18.1[a]
→3)-α-D-Rha*p*-(1→ (C)	97.7	68.2	76.6	71.7	70.2	18.2[a]
Calculated data for structure 1						
→3)-β-L-Rha*p*-(1→ (A)	101.4	68.3	78.2	71.4	73.2	18.0
→4)-α-L-Rha*p*-(1→ (B)	97.6	71.6	70.3	83.8	69.0	18.0
→3)-α-D-Rha*p*-(1→ (C)	97.6	68.3	75.5	71.8	70.0	18.0

[a] Assignments could be interchanged.

ride was interpreted completely by using heteronuclear ^{13}C–^{1}H shift-correlated spectroscopy (XHCORRD), and the results are given in Table II. The relatively low-field positions of signals for C-4 of unit B at 84.0 p.p.m. and units A and C at 78.2 and 76.6 p.p.m., respectively, confirmed the 4-substitution of unit B and the 3-substitution of units A and C.

These positions of substitution were confirmed by methylation analysis, which gave 1,3,5-tri-*O*-acetyl-2,4-di-*O*-methylrhamnitol and 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylrhamnitol in the ratio ~2:1.

In order to determine the absolute configurations of units A–C and to confirm the structure of the polysaccharide, the computer-assisted ^{13}C-n.m.r.-based method[5] was applied. This method involves the evaluation of the spectra for all possible structures of a linear polysaccharide with the given monosaccharide composition, and comparison of the calculated and experimental spectra. The analysis revealed structure **1**, which was characterized by the smallest sum of the squared deviations of the chemical shifts in the calculated and experimental spectra (S = 0.6 per sugar residue).

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

1

$$\rightarrow 3)\text{-}\beta\text{-L-Rha}p\text{-}(1\rightarrow 3)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow 4)\text{-}\alpha\text{-D-Rha}p\text{-}(1\rightarrow$$

2

This structure was consistent with the data given above, and the assignment of the ^{13}C signals accorded with that based on the XHCORRD method. Another possible structure (**2**) of the polysaccharide was characterised by a considerably larger value (2.3) of S; although the positions of substitution of the sugar residues were the same as in **1**, structure **2** did not agree with the n.O.e. data which showed, in particular, that the β-rhamnose residue was attached at position 4 (not 3). All other possible structures of the polysaccharide had S values of >3.

It is noteworthy that calculations based on the assumption that all of the rhamnose residues had the same absolute configuration led to no structure with a value of S of <7 and thus confirmed the sensitivity of ^{13}C chemical shifts in oligo- and poly-saccharides to the absolute configurations of the constituent monosaccharides[5,6].

Thus, on the basis of the non-destructive analysis, it was concluded that the O-specific polysaccharide of *P. syringae* pv. *lachrymans* strain 7591 has structure **1**. As in other representatives of the *P. syringae* group, this O-antigen is composed mainly of rhamnose, but its unusual feature is the presence of both the D and L enantiomers of this sugar, which appears not to have been observed hitherto in bacterial polysaccharides. Also, the O-antigen of *P. syringae* pv. *lachrymans* 7591, which belongs to the serogroup IX, includes a β-rhamnose residue and thus differs from the O-antigens of other *P. syringae*-related phytopathogenic pseudomonads that belong to serogroups I–VII (ref. 1 and papers cited therein), which contain only α-rhamnose residues.

EXPERIMENTAL

General methods. — 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 chromatographic conditions as in g.l.c. Gel-permeation chromatography was performed on a column (3.5 x 70 cm) of Sephadex G-50 in pyridine acetate buffer (pH 5.5). Fractions were analysed by the phenol–sulfuric acid reaction.

The growth of bacteria and the isolation of the lipopolysaccharide and the O-specific polysaccharide were performed as described[2,7].

Samples were hydrolysed with 2M trifluoroacetic acid in sealed ampoules for 2 h at 120°. Methylation analysis was performed according to the Hakomori procedure[8] and the products were recovered using a Sep-Pak cartridge[9].

N.m.r. spectroscopy. — The ^{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). The ^1H-n.m.r. and n.O.e. spectra were recorded with a Bruker WM-250 instrument for solutions in D_2O at 30° (internal acetone, δ 2.23).

The standard Bruker software was used to obtain heteronuclear ^{13}C–^1H COSY spectrum (XHCORRD) under the following conditions: 90° pulse of 25 μs for ^1H and 14 μs for ^{13}C, the time domain in f_2 was 2K, 128 spectra were collected with 1000 scans, the spectral window was 4200 Hz in the f_2 domain and 740 Hz in the f_1 domain (the region for the resonances of ring carbons and protons only), the relaxation delay D1 was 0.8 s, and D3 and D4 were 3.2 μs and 1.6 μs, respectively (optimal for $^1J_{C,H} \sim 150$ Hz). Fourier transformation was performed with an unshifted sine-bell function in both dimensions.

1D-N.O.e. spectra were obtained, using the Bruker NOEMULT program, by the difference mode where the on-resonance irradiated spectrum was substracted from that in which the irradiation frequence was off-resonance.

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