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Note

Structural studies of the O-specific side-chain of lipopolysaccharide from *Burkholderia gladioli* pv. *gladioli* strain NCPPB 1891

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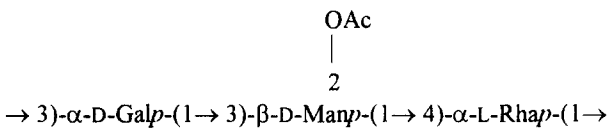
Abstract

A polymeric fraction (the O-antigenic side-chain) has been isolated from the lipopolysaccharide of *Burkholderia gladioli* pv. *gladioli* strain NCPPB 1891 after mild acid hydrolysis. The components of the polymer and their molar proportions were L-Rha (1), D-Gal (1), D-Man (1), and *O*-acetyl (1). By means of chemical degradations and NMR studies, the repeating unit of the polymer was shown to be a linear trisaccharide of the structure shown.



Keywords: *Burkholderia gladioli*; Lipopolysaccharide; O-antigen

1. Introduction

Most of the organisms in the *Pseudomonas* rRNA homology group II have recently been renamed *Burkholderia* spp. [1]. The group comprises organisms having potential virulence factors, including lipopolysaccharide (LPS) and polar lipids, which make structural studies of interest. It has been shown that the lipids of the *Burkholderia* spp. are unusual in that they include two forms of phosphatidylethanolamine and of an ornithine amide lipid, differing in the presence or absence of a 2-hydroxy acid [1,2], and that *B. caryophylli* LPS contains some very unusual sugars [3–5]. Work has been carried out on the involvement of *B. gladioli* as the causal agent in various plant diseases (e.g. onion rot [6] and mushroom rot [7]) and in recent years as a human pathogen. *B. cepacia*, the type species of the genus, has long been known as a coloniser of the lungs of cystic fibrosis (CF) patients [8,9], but more recently *B. gladioli* has also been isolated from the respiratory tracts of CF sufferers [10], and in some cases isolates

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possessing characteristics of both *B. cepacia* and *B. gladioli* have been reported [11]. *B. gladioli* has also been reported as the cause of infection in sufferers of chronic granulomatous disease [12–14]. A structure for the O-specific side-chain of *B. gladioli* pv. *alli-icola* strain 8494 has been published [15], but no structure for *B. gladioli* pv. *gladioli* has yet been established. Here we report the structure of the O-antigen from strain NCPPB 1891, the type strain of this pathovar.

2. Results and discussion

LPS was obtained by hot, aqueous phenol extraction of the defatted cell walls of *B. gladioli* NCPPB 1891 (yield, 19%). During mild acid hydrolysis of the LPS the suspension became dark brown, possibly due to the release and decomposition of 4-amino-4-deoxyarabinose, as observed in earlier studies of LPS from *B. cepacia* [16], although the loss of a labile substituent from the O-antigen cannot be excluded. The yield of the acid-stable polymeric fraction, isolated by gel permeation chromatography, was 52%. The main monosaccharide components of the polymer were identified as L-Rha, D-Gal, and D-Man in approximate molar ratios 1:1:1; small amounts of Glc and heptose were also present. Methylation analysis of the polymer showed that (a) the aldohexose residues were pyranoid, (b) the Gal*p* and Man*p* were 3-substituted, and (c) the Rha residue occurred as 4-substituted Rha*p* or 5-substituted Rha*f*. The results of NMR studies (vide infra) confirmed the inference that the polymer had a regular linear structure based on a trisaccharide repeating unit, and also revealed that each unit carried an *O*-acetyl substituent (δ_H 2.21; δ_C 174.38 and 21.23).

The ^{1}H NMR spectrum of the polymeric fraction (recorded at 70 °C, see Fig. 1) contained four signals (each 1 H) in the anomeric region at δ 5.53 (d, J 2.4 Hz), 5.28 ($J_{1,2}$ 3.8 Hz), 5.09 (unresolved), and 5.00 (unresolved), of which only the last three correlated (HMQC) with anomeric signals in the ^{13}C NMR spectrum, at δ 101.22, 100.03, and 102.53, respectively. A COSY spectrum showed that the signal with δ_H 5.53 (which correlated with δ_C 73.21) corresponded to H-2 of the residue giving the anomeric signals at δ_H 5.09 and δ_C 100.03. The downfield location of the signal for H-2 is consistent with regiospecific *O*-acetylation at position 2 of this residue (Man or Rha; the value of $J_{1,2}$ for the anomeric signal with δ_H 5.28 showed that it was derived from α-Gal*p*).

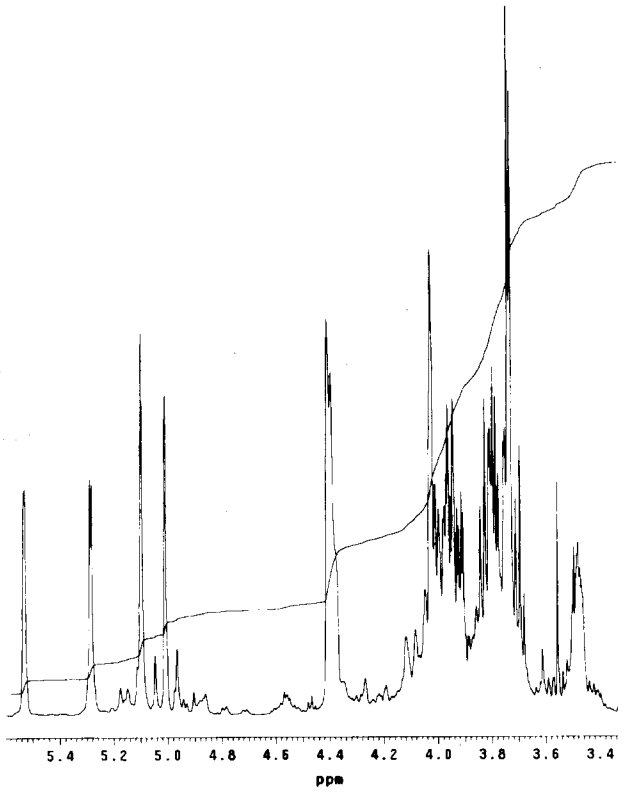


Fig. 1. ^{1}H NMR spectrum of the original (*O*-acetylated) polymer. The spectrum for the sample in ^2H_2O was recorded at 600 MHz and 70 °C with acetone (δ_H 2.22) as internal reference. In addition to the signals shown the spectrum contained a methyl signal (d, δ 1.32).

As expected, the ^{1}H NMR spectrum of the *O*-deacetylated polymer (recorded at 40 °C) contained only three anomeric signals, at δ 5.28 ($J_{1,2}$ 3.4 Hz), 5.03 (unresolved), and 4.91 (unresolved), and a signal at δ 1.34 ($J_{5,6}$ 6.0 Hz) for Rha H-6, inter alia. The corresponding ^{13}C NMR spectrum (Fig. 2) contained 18 discrete signals, including anomeric signals at δ 102.92, 101.46, and 101.22, signals for two unsubstituted hydroxymethyl carbons (δ 61.92 and 61.81), and Rha C-6 (δ 17.78). Most of the signals in both NMR spectra, including the ones of greatest diagnostic value, could be assigned (Table 1) with the aid of COSY, relayed COSY, TOCSY, NOESY, and HMQC spectra. For this purpose the sugar residues were coded **A**, **B**, and **C**, in order of decreasing chemical shift for the anomeric signals in the ^{1}H NMR spectrum. Residue **A** was identified as α-Gal*p* from the value of $J_{1,2}$, and 3-substitution was inferred from the downfield location of the signal for C-3 compared with the corresponding signal for free α-Gal*p* [17]. Residue **B** was identified as Rha*p* from the spin system leading from H-1 to H-6: the α configuration was assigned from the chemical shift for H-5 [18],

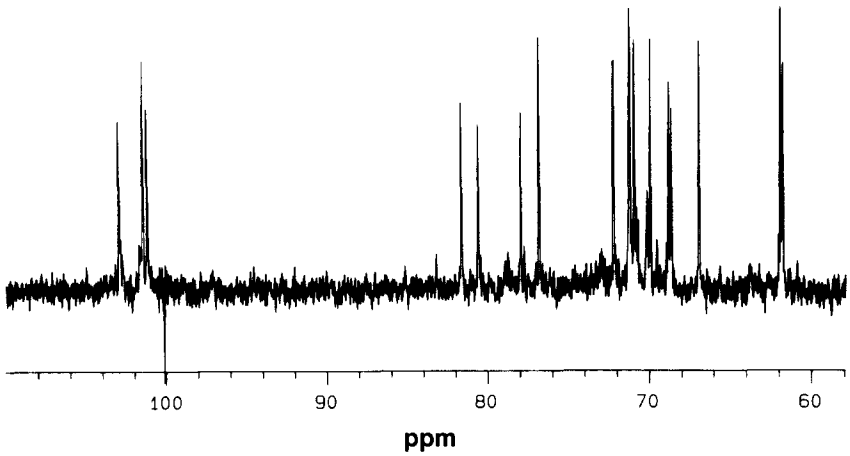


Fig. 2. ^{13}C NMR spectrum of the *O*-deacetylated polymer. The spectrum for the sample in $^{2}H_2O$ was recorded at 150 MHz and 40 °C with acetone (δ_C 31.07) as internal reference. In addition to the signals shown the spectrum contained a methyl signal (δ 17.78).

and substitution at position 4 was apparent from the downfield glycosylation effect. Similarly, the spectral assignments for residue **C**, particularly for C-3, H-5, and C-5, were consistent only with those expected for 3-substituted β-Man*p*.

Examination of the NOESY spectrum established the order of the sugar residues in the repeating unit. The inter-residue contacts between H-1 of **A** and H-3 of **C**, H-1 of **B** and H-3 of **A**, and H-1 of **C** and H-4 of **B** provided the sequence → **A** → **C** → **B** → and structure **1** for the repeating unit of the *O*-deacetylated polymer. Intra-residue contacts with H-2 (for **A** and **B**), and with H-2, H-3, and H-5 (for **C**) confirmed the anomeric configurations shown. Support for the proposed structure was also provided by a Smith degradation. Methylation analysis of the oligomeric product (SD1) gave the products from unsubstituted and 3-substituted hexopyranose residues, which were identified as the derivatives from Gal*p* and Man*p*, respectively, by comparison with standards (GLC).

A **C** **B**

→ 3)-α-D-Gal*p*-(1→ 3)-β-D-Man*p*-(1→ 4)-α-L-Rha*p*-(1→

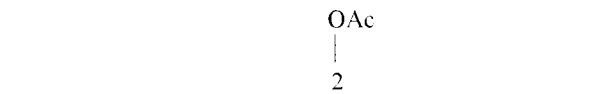
1

Table 1
NMR data [a] for *O*-deacetylated polymer

Atom		Residue		
		→ 3)-α-Gal*p*-(1 → **A**	→ 4)-α-Rha*p*-(1 → **B**	→ 3)-β-Man*p*-(1 → **C**
1	H	5.28 (5.28)	5.03 (5.00)	4.91 (5.09)
	C	101.46 (101.22)	102.92 (102.53)	101.22 (100.03)
2	H	3.94 (3.94)	~ 4.06 (4.02)	4.28 (5.53)
	C	68.81	71.20 (71.31)	71.27 (73.21)
3	H	4.01	3.97 (3.91)	3.76 (3.98)
	C	77.99	70.93 (70.99)	81.69 (78.20)
4	H	~ 4.06	3.68	3.77
	C	69.94	80.61	66.93
5	H	4.12	~ 3.89	3.40
	C	72.22	68.64	76.84
6	H	~ 3.74	1.34	~ 3.93, ~ 3.76
	C	61.92	17.78	61.81

[a] Spectra for the *O*-deacetylated polymer in $^{2}H_2O$ were obtained at 40 °C and 600 MHz (^{1}H) or 150 MHz (^{13}C). Spectra for the original polymer (data in parentheses) were obtained at 70 °C. Values for chemical shift are given relative to internal acetone (δ_H set at 2.22 and δ_C at 31.07).

As noted earlier, NMR data for the original polymer pointed to regiospecific *O*-acetylation at position 2 of a sugar residue with the *manno* configuration. The actual location of the substituent was determined by further examination of the NMR spectra. *O*-Deacetylation caused a marked downfield shift of one of the four signals corresponding to the positions of glycosylation or to C-5 of Man*p* (δ 81.32, 78.20, 77.24, and 77.11 for the original polymer; δ 81.69, 80.61, 77.99, and 76.84 for the *O*-deacetylated polymer). This shift must be a β effect on C-3 of the acetylated residue, which must therefore correspond to Man*p*. Although spectral overlap limited the extent of signal assignment, the partial data shown (Table 1) confirm acetylation of O-2 of Man*p*. Thus, the chemical shifts for Gal*p* are almost identical in both the parent and the *O*-deacetylated polymer, confirming the previous inference that Gal is not the site of acetylation. Likewise, the values for Rha*p* vary only slightly with acetylation. By contrast, the signal for H-2 of Man*p* has δ 4.28 in the spectrum for the *O*-deacetylated product but δ 5.53 in that of the parent polymer, while the corresponding values for C-2 are δ 71.27 and 73.21, respectively. The upfield shift for C-2 on deacetylation, and the accompanying downfield shifts for C-1 and C-3, establish the location of the acetyl group at position 2 (structure **2**).



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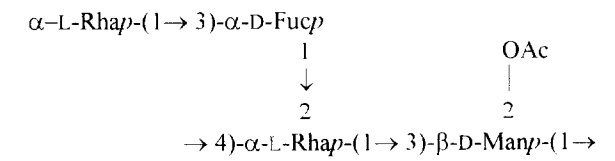
The structure of the repeating unit in the O-antigen of *B. gladioli* pv. *gladioli* NCPPB 1891 does not seem to have been reported previously, although similar trisaccharide units of Gal, Man, and Rha incorporating the disaccharide element → **B** → **A** → form the backbone in many O-antigens of salmonellae [19]. Also, it is noteworthy that in the O-antigen of *B. gladioli* pv. *alliicola* 8494, which has the branched tetrasaccharide repeating unit of structure **3**, the disaccharide backbone is constructed from residues **B** and **C**, the latter again with a 2-*O*-acetyl substituent [15].



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3. Experimental

Growth of bacteria, isolation and fractionation of the LPS.—*B. gladioli* pv. *gladioli* strain NCPPB 1891 was grown in Nutrient Broth No. 2 (Oxoid, 20 L) for 27 h at 37 °C, aerated at 20 L min^{-1}, and stirred at 300 rpm. The wet cells (yield, 86 g) were disintegrated and the cell walls were isolated (yield, 3.5 g) and defatted, and the LPS was extracted with hot aq phenol (45%, w/w; 68 °C, 15 min) as in earlier studies [20,21] (yield, 670 mg). Fractionation of the LPS was achieved by mild acid hydrolysis (aq 1% HOAc, 100 °C, 1.5 h) followed by gel filtration of the water-soluble products on Sephadex G-50 in pyridine–HOAc buffer (pH 5.4). The eluate was monitored for total carbohydrate by the phenol–H_2SO_4 method [22].

NMR spectroscopy.—Spectra (1D and 2D) for products (the original and *O*-deacetylated polymer, and the Smith degradation product SD1) in D_2O were recorded with Jeol JNM-GX270 and Varian DXR600S spectrometers. The NMR spectra were recorded at 40 °C (*O*-deacetylated product), or at 70 °C (original polymer and SD1) with acetone (δ_H 2.22, δ_C 31.07) as the internal reference in both cases. Standard pulse sequences were used to obtain COSY, relayed COSY, HMQC, NOESY, and TOCSY spectra.

Determination of monosaccharide composition.—Samples were hydrolysed with 2 M CH_3COOH at 98 °C for 16 h [23]. Products were analysed by PC (13:5:4 EtOAc–pyridine–water), high-performance anion-exchange chromatography (Dionex DX300, CarboPac PA100 column, 0.18 M NaOH), and by GLC of the alditol acetates. Absolute configurations were assigned by GLC of the but-2-yl glycoside acetates [24] and by enzymic assay [25,26].

Chemical degradations.—*O*-Deacetylation of the original polymer was carried out by treatment with 0.1 M NaOH at room temperature for 16 h followed by neutralisation with Dowex 50 (H^+) resin. Smith degradation was carried out by oxidation of the polymer (50 mM $NaIO_4$, 4 °C, 4 days) followed by reduction ($NaBH_4$), dialysis, and hydrolysis with 1 M CH_3COOH (room temperature, 16 h). Standard procedures were used for methylation analysis [27–29]. The results were monitored using GLC and GLC–MS.

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References

[1] E. Yabuuchi, Y. Kosako, H. Oyaizu, I. Yano, H. Hotta, Y. Hashimoto, T. Ezaki, and M. Arakawa, *Microbiol. Immunol.*, 36 (1992) 1251–1275.
[2] L. Galbraith and S.G. Wilkinson, *J. Gen. Microbiol.*, 137 (1991) 197–202.
[3] M. Adinolfi, M.M. Corsaro, C. De Castro, R. Lanzetta, M. Parilli, A. Evidente, and P. Lavermicocca, *Carbohydr. Res.*, 267 (1995) 307–311.
[4] M. Adinolfi, M.M. Corsaro, C. De Castro, A. Evidente, R. Lanzetta, A. Molinaro, and M. Parilli, *Carbohydr. Res.*, 284 (1996) 111–118.
[5] M. Adinolfi, M.M. Corsaro, C. De Castro, A. Evidente, R. Lanzetta, P. Lavermicocca, and M. Parilli, *Carbohydr. Res.*, 284 (1996) 119–133.
[6] P.J. Wright, R.G. Clark, and C.N. Hale, *N. Z. J. Crop Horticult. Sci.*, 21 (1993) 225–227.
[7] P.T. Atkey, T.R. Fermor, and S.P. Lincoln, *Mycol. Res.*, 96 (1992) 717–722.
[8] J.R.W. Govan, J.E. Hughes, and P. Vandamme, *J. Med. Microbiol.*, 45 (1996) 395–407.
[9] J.R.W. Govan and V. Deretic, *Microbiol. Rev.*, 60 (1996) 539–574.
[10] J.C. Christenson, D.F. Welch, G. Mukwaya, M.J. Muszynski, R.E. Weaver, and D.J. Brenner, *J. Clin. Microbiol.*, 27 (1989) 270–283.
[11] I.N. Simpson, J. Finlay, D.J. Winstanley, N. Dewhurst, J.W. Nelson, S.L. Butler, and J.R.W. Govan, *J. Antimicrob. Chemother.*, 34 (1994) 353–361.
[12] J.P. Ross, S.M. Holland, V.J. Gill, E.S. DeCarlo, and J.I. Gallin, *Clin. Infect. Dis.*, 21 (1995) 1291–1293.
[13] C.A. Smith, K. Sullivan, N.A. Pawlowski, J.M. Greene, T. Brown, A.G. Yip, J. Rae, J. Curnutte, and S.D. Douglas, *J. Allergy Clin. Immunol.*, 97 (1996) 874.
[14] B. Hoen, *Clin. Infect. Dis.*, 23 (1996) 411.
[15] E.V. Vinogradov, E.D. Daeva, A.S. Shashkov, Y.A. Knirel, G.M. Zdorovenko, L.M. Yakovleva, N.Y. Gubanova, and L.P. Solyanik, *Carbohydr. Res.*, 212 (1991) 313–320.
[16] A.D. Cox and S.G. Wilkinson, *Mol. Microbiol.*, 5 (1991) 641–646.
[17] P.-E. Jansson, L. Kenne, and G. Widmalm, *Carbohydr. Res.*, 188 (1989) 169–191.
[18] A. Adeyeye, P.-E. Jansson, B. Lindberg, S. Abaas, and S.B. Svenson, *Carbohydr. Res.*, 176 (1988) 231–236.
[19] Y.A. Knirel and N.K. Kochetkov, *Biochemistry (Moscow)*, 59 (1994) 1325–1383.
[20] O. Westphal and K. Jann, *Methods Carbohydr. Chem.*, 5 (1965) 83–91.
[21] D. Oxley and S.G. Wilkinson, *Carbohydr. Res.*, 195 (1989) 111–115.
[22] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.
[23] C.C. Yu Ip, V. Manam, R. Hepler, and J.P. Hennessey, *Anal. Biochem.*, 201 (1992) 343–349.
[24] G.J. Gerwig, J.P. Kamerling, and J.F.G. Vliegenthart, *Carbohydr. Res.*, 77 (1979) 1–7.
[25] D. Oxley and S.G. Wilkinson, *Carbohydr. Res.*, 187 (1989) 295–301.
[26] S.R. Haseley and S.G. Wilkinson, *Eur. J. Biochem.*, 237 (1996) 229–233.
[27] B. Lindberg and J. Lönngren, *Methods Enzymol.*, 50C (1978) 3–33.
[28] L.R. Phillips and B.A. Fraser, *Carbohydr. Res.*, 90 (1981) 149–152.
[29] A.J. Mort, S. Parker, and M.S Kuo, *Anal. Biochem.*, 133 (1983) 380–384.