Carbohydrate Research, 247 (1993) 249–254
Elsevier Science Publishers B.V., Amsterdam

Structure of the O19 antigen of *Xanthomonas maltophilia*

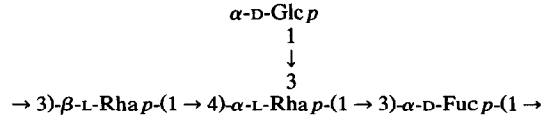
Angela M. Winn [a], Lesley Galbraith [a], Graham S. Temple [b] and Stephen G. Wilkinson [a]

[a] *School of Chemistry, University of Hull, Hull HU6 7RX (United Kingdom)*
[b] *Public Health Laboratory, Hull Royal Infirmary, Hull HU3 2JZ (United Kingdom)*

(Received January 22nd, 1993; accepted March 13th, 1993)

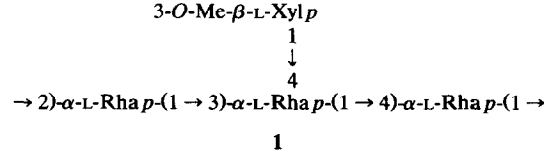
ABSTRACT

The O-specific polymer from a strain of *Xanthomonas maltophilia* O19 contains D-glucose, L-rhamnose, and D-fucose. By means of chemical degradations and NMR studies, the repeating unit of the polymer was determined to be a branched tetrasaccharide of the structure shown.



INTRODUCTION

The growing importance of *Xanthomonas (Pseudomonas) maltophilia* as an opportunistic pathogen[1–6] has led to the development of a scheme for typing clinical isolates by their heat-stable (lipopolysaccharide) O antigens[7]. So far, structures of the repeating units have only been reported for polymers from three strains: structure **1** (refs. 8, 9) for the type strain NCTC 10257 (O8), **2** (ref. 10) for strain NCIB 9204 (O1), and **3** (ref. 11) for strain 555 (O12/O27). Even these limited data show the presence in the O antigens from *X. maltophilia* of unusual components and structural features. We now report the structure of the repeating unit in the O-specific polymer from a strain belonging to serogroup O19, one of the most common in a survey of clinical and environmental isolates[7].



α-D-Araf
1
↓
6
→ 3)-β-D-Glcp NAc-(1 → 3)-α-L-6dTalp-(1 →

2

α-D-Fucp3NAc
1
↓
2
→ 3)-α-D-Rhap4NAc-(1 → 3)-α-D-RhapNAc-(1 → 3)-β-D-Rhap-(1 → 3)-α-D-RhapNAc-(1 →

3

RESULTS AND DISCUSSION

Lipopolysaccharide was extracted from defatted, isolated cell walls of *X. maltophilia* strain 70401 (yield, 21%). Of the water-soluble material produced by mild acid hydrolysis of the lipopolysaccharide (yield, 62%), ~ 80% was recovered as O-specific polymer after fractionation on Sephadex G-50. The monosaccharide components were identified as L-rhamnose, D-fucose, and D-glucose (relative peak areas in GLC of the alditol acetates, 2.00 : 1.10 : 0.87). The NMR spectra of the polymer confirmed the presence of a tetrasaccharide repeating-unit incorporating three 6-deoxyhexose residues. Thus, the ^{1}H NMR spectrum contained signals (each 1 H) for anomeric protons at δ 5.14 ($J_{1,2}$ 3.7 Hz), 5.09 (unresolved), 5.07 ($J_{1,2}$ 3.8 Hz), and 4.73 (unresolved) and methyl singlets at δ 1.38 ($J_{5,6}$ 5.9 Hz), 1.33 ($J_{5,6}$ 6.3 Hz), and 1.19 ($J_{5,6}$ 6.5 Hz). The inference that one rhamnose residue was β-linked and the other three sugar residues were α-linked was confirmed by the ^{13}C NMR spectrum (Fig. 1). The spectrum contained 23 discrete signals (one of double intensity), including signals for anomeric carbons of pyranosyl residues at δ 102.58 ($^1J_{CH}$ 171 Hz), 101.46 ($^1J_{CH}$ 161 Hz), 96.59 ($^1J_{CH}$ 171 Hz), and 95.06 ($^1J_{CH}$ 170 Hz), signals for three methyl groups (δ 18.15, 17.73, and 15.96), and one for an unsubstituted hydroxymethyl carbon (δ 61.48).

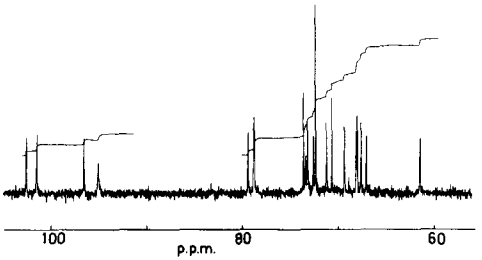
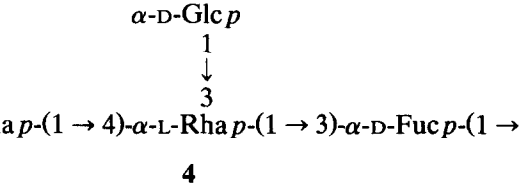


Fig. 1. ^{13}C NMR spectrum of the native polymer. The spectrum for the sample in D_2O was recorded at 100.62 MHz and 40°C with acetone (δ 31.07) as the internal reference. In addition to the signals shown, the spectrum contained others at δ 18.15, 17.73, and 15.96.

Methylation analysis of the polymer gave four products, derived from an unsubstituted hexopyranosyl residue, two different 3-substituted 6-deoxyhexopyranosyl residues, and a 3,4-disubstituted 6-deoxyhexopyranosyl residue. Thus, the tetrasaccharide repeating-unit is branched, with rhamnose at the branch and glucose as the terminal sugar. Partial acid hydrolysis of the polymer gave an α-glucosylrhamnose which was shown by the following studies to correspond to these two sugar residues. Smith degradation of the native polymer gave a linear glycan (SD1) lacking glucose, showing that the backbone of the O19 antigen was a repeating trisaccharide of rhamnose (2) and fucose (1) residues. The ^{1}H NMR spectrum of SD1 included signals for anomeric protons at δ 5.07 ($J_{1,2} \sim 4$ Hz), 5.05 (unresolved), and 4.72 (unresolved). As expected, the ^{13}C NMR spectrum of SD1 contained 17 discrete signals (one of double intensity), including signals for anomeric carbons at δ 102.84, 101.19, and 96.65. These data identify glucose as the origin of the signals with δ_H 5.14 and δ_C 95.06 in the spectra of the native polymer. This inference was confirmed by a heteronuclear shift-correlation experiment. Methylation analysis of SD1 showed the presence of a 4-substituted rhamnose residue in place of the 3,4-disubstituted one.

A second Smith degradation was carried out, yielding the oligomeric product SD2. The ^{1}H NMR spectrum of SD2 contained signals for anomeric protons at δ 5.11 ($J_{1,2} \sim 4$ Hz) and 4.80 ($J_{1,2} \sim 1$ Hz), showing that the 4-substituted rhamnose residue in SD1 was α-linked, and methyl signals at δ 1.39 (3 H) and 1.25 (6 H). Further methylation analysis of SD2 showed that the 3-substituted rhamnose residue was still present and that fucose was at the non-reducing terminus. These results permit structure **4** to be assigned to the repeating unit of the original polymer. The sequence of the two rhamnose residues was confirmed by NOE difference spectra for both the native polymer and SD1. Thus, for SD1, irradiation at δ 4.72 (H-1 of β-Rha) showed a strong inter-residue effect at δ 3.61 (H-4 of α-Rha). Assignments of the NMR signals for the native polymer and SD1 are given in Tables I and II, respectively.



4

Compared with other O antigens of *X. maltophilia* studied to date, the O19 antigen is rather unremarkable in composition and structure. Although D-fucose is uncommon compared with the L isomer, it is also present in the O3 antigen of *X. maltophilia*[12].

EXPERIMENTAL

Growth of bacteria, and isolation and fractionation of the lipopolysaccharide.— Strain 70401 (isolated from a catheter specimen of a patient with a suspected

TABLE I

NMR data [a] for the native O19 polymer

Atom		Residue			
		α-Glc*p*-(1→	⇉3,4-)-α-Rha*p*-(1→	→3)-α-Fuc*p*-(1→	→3)-β-Rha*p*-(1→
1	H	5.14	5.09	5.07	4.73
	C	95.06	102.58	96.59	101.46
2	H	3.58	4.30	~3.94	4.21
	C	72.38	67.09	68.01	68.13
3	H	~3.95	4.13	4.00	3.64
	C	73.40	73.64	78.70	~78.8
4	H	3.44	3.79	3.89	3.49
	C	70.66	79.36	72.61	71.24
5	H	~4.16	~3.96	4.32	3.41
	C	72.38	69.38	67.62	73.21
6	H	3.74	1.33	1.19	1.38
	C	61.48	17.73	15.96	18.15
6′	H	3.85			

[a] Values for chemical shifts relative to acetone (δ_H 2.22; δ_C 31.07).

urinary tract infection) was grown for 16 h at 37°C in Nutrient Broth No. 2 (Oxoid, 20 L) with aeration at 20 L min^{-1}. The wet cells (200 g) were disintegrated mechanically[13] to provide isolated walls (dry weight, 7.33 g). Lipopolysaccharide (1.43 g) was extracted from the defatted cell walls by the aqueous-phenol method[8].

TABLE II

NMR data [a] for the first Smith-degradation product (SD1)

Atom		Residue		
		→4)-α-Rha*p*-(1→	→3)-α-Fuc*p*-(1→	→3)-β-Rha*p*-(1→
1	H	5.05	5.07	4.72
	C	102.84	96.65	101.19
2	H	4.14	~3.95	4.26
	C	70.41	68.04	68.20
3	H	3.97	3.99	3.66
	C	69.87	78.58	78.58
4	H	3.61	3.88	3.52
	C	83.55	72.59	70.98
5	H	3.92	4.32	3.50
	C	68.38	67.70	73.10
6	H	1.30	1.19	1.36
	C	17.35	15.95	17.65

[a] Values for chemical shifts relative to acetone (δ_H 2.22; δ_C 31.07).

The O-specific polymer was obtained by hydrolysis of the lipopolysaccharide with aq 1% acetic acid for 1.75 h at 100°C, followed by chromatography of the water-soluble products on Sephadex G-50.

General methods.—NMR spectra were recorded with a Bruker WH-400 spectrometer (native polymer and product SD1) or with a JEOL JNM-GX270 spectrometer (other products). In the former case, spectra were obtained at 70°C (^{1}H) or 40°C (^{13}C) with acetone (δ_H 2.22, δ_C 31.07) as the internal reference. Standard pulse sequences were used for 2D spectra (C–H shift correlation, COSY, and relayed COSY). Other ^{1}H NMR spectra (270 MHz) were obtained at 70°C with sodium 4,4-dimethyl-4-silapentane-1-sulphonate as the external reference. GLC separations of alditol acetates, methylated alditol acetates, oligosaccharide-alditols, and but-2-yl glycoside acetates were carried out with a fused-silica capillary column (BP1) fitted to a Carlo Erba Mega 5160 chromatograph. GLC–MS was carried out with a Finnigan model 1020B instrument. HPLC separations of mono- and oligo-saccharides were achieved using Gilson equipment and columns of TSKgel G-Oligo-PW (Anachem) or HPX-87P (Bio-Rad). CD spectra were provided by the Chiroptical Spectroscopy Service (University of London). Polarimetric measurements were made with an AA-10 instrument (Optical Activity Ltd.).

Determination of sugar composition.—Samples were hydrolysed with 2 M HCl at 105°C for 2 h (followed by neutralisation and deionisation with resins), or with 2 M trifluoroacetic acid at 105°C for 16 h (followed by repeated rotary evaporation). Monosaccharides were identified by PC with ethyl acetate–pyridine–water (13:5:4) as the solvent, by GLC of the alditol acetates, by HPLC on HPX-87P (eluted with water), and by their ^{1}H NMR spectra. Individual monosaccharides were isolated by preparative PC and purified by HPLC. Absolute configurations were established by GLC of the but-2-yl glycoside acetates[14] (fucose and rhamnose), by polarimetry of the free sugar (fucose; $[\alpha]_D$ +75°), by the CD curve for the alditol acetate[15] (rhamnose), and by assay with D-glucose oxidase (EC 1.1.3.4).

Structural methods.—Methylation analyses followed standard procedures[16–18]. In Smith degradations, samples were oxidised with 50 mM sodium periodate at 4°C for several days. After reduction ($NaBH_4$ or $NaBD_4$) and workup, the products were treated with M trifluoroacetic acid at 20°C overnight, then fractionated on Sephadex G-50 or G-15.

Partial acid hydrolysis of the native polymer was carried out with 0.1 M trifluoroacetic acid at 100°C for periods between 14 min and 2 h; the process was monitored by HPLC. A disaccharide was isolated from the 2-h hydrolysate by successive HPLC on TSKgel G-Oligo-PW (eluted with 0.2 M sodium acetate at 1.0 mL min^{-1}) and HPX-87P (eluted with water at 70°C and 0.6 mL min^{-1}). The ^{1}H NMR spectrum included signals for anomeric protons at δ 5.21 (0.6 H) and 4.91 (0.4 H) (both partially resolved), and others at δ 5.16 (0.4 H) and 5.14 (0.6 H) (both with $J_{1,2} \sim 4$ Hz). After reduction ($NaBH_4$), the spectrum showed only one anomeric proton with δ 5.25 ($J_{1,2} \sim 4$ Hz) and a methyl group with δ 1.31 ($J_{5,6} \sim 6$ Hz). Total acid hydrolysis of the disaccharide gave glucose and rhamnose.

ACKNOWLEDGMENTS

We thank Dr. B. Schable (Centers for Disease Control, Atlanta) for the serological typing, the S.E.R.C. for an allocation on the high-field NMR service at the University of Warwick, and Beaulah Banfield of the Chiroptical Spectroscopy Service at Birkbeck College, University of London (for the CD measurements). Instrumental services were also provided by our colleagues Mrs. B. Worthington, Mr. R. Knight, Mr. A.D. Roberts, and Dr. D.F. Ewing.

REFERENCES

1 P.E. Schoch and B.A. Cunha, *Infect. Control*, 8 (1987) 169–172.
2 W.F. Marshall, M.R. Keating, J.P. Anhalt, and J. Steckelberg, *Mayo Clin. Proc.*, 64 (1989) 1097–1104.
3 M. Akova, G. Bonfiglio, and D.M. Livermore, *J. Med. Microbiol.*, 35 (1991) 208–213.
4 P. Hohl, R. Frei, and P. Aubry, *Diagn. Microbiol. Infect. Dis.*, 14 (1991) 447–450.
5 W. Cullmann, *Chemotherapy*, 37 (1991) 246–250.
6 M. Lesco-Bornet, J. Pierre, D. Sarkis-Karam, S. Lubera, and E. Bergogne-Berezin, *Antimicrob. Agents Chemother.*, 36 (1992) 669–671.
7 B. Schable, D.L. Rhoden, R. Hugh, R.E. Weaver, N. Khardori, P.B. Smith, G.P. Bodey, and R.L. Anderson, *J. Clin. Microbiol.*, 27 (1989) 1011–1014.
8 D.J. Neal and S.G. Wilkinson, *Carbohydr. Res.*, 69 (1979) 191–201.
9 D.J. Neal and S.G. Wilkinson, *Eur. J. Biochem.*, 128 (1982) 143–149.
10 S.G. Wilkinson, L. Galbraith, and W.J. Anderton, *Carbohydr. Res.*, 112 (1983) 241–253.
11 J.L. DiFabio, M.B. Perry, and D.R. Bundle, *Biochem. Cell Biol.*, 65 (1987) 968–977.
12 C.T. Miles and S.G. Wilkinson, unpublished results.
13 C.J. Brigden and S.G. Wilkinson, *Carbohydr. Res.*, 138 (1985) 267–276.
14 D. Oxley and S.G .Wilkinson, *Carbohydr. Res.*, 204 (1990) 85–91.
15 G.M. Bebault, J.M. Berry, Y.M. Choy, G.G.S. Dutton, N. Funnell, L.D. Hayward, and A.M. Stephen, *Can. J. Chem.*, 51 (1973) 324–326.
16 B. Lindberg and J. Lönngren, *Methods Enzymol.*, 50C (1978) 3–33.
17 L.R. Phillips and B.A. Fraser, *Carbohydr. Res.*, 90 (1981) 149–152.
18 A.J. Mort, S. Parker, and M.-S. Kuo, *Anal. Biochem.*, 133 (1983) 380–384.