Carbohydrate Research, 198 (1990) 153–156
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Structure of the O-specific polymer for *Pseudomonas cepacia* serogroup O7

ANDREW D. COX AND STEPHEN G. WILKINSON
School of Chemistry, The University, Hull HU6 7RX (Great Britain)
(Received May 26th, 1989; accepted for publication, July 18th, 1989)

Schemes for the serological typing of *Pseudomonas cepacia* by its heat-stable (O) antigens have been developed[1–3] in order to assist in the epidemiological monitoring of clinical isolates of this opportunistic pathogen. The results of recent structural studies by Knirel and co-workers[4–10] and ourselves[11,12] on the putative O antigens of *P. cepacia* are providing a chemical basis for the serological classification and relationships. We have used the reference strains selected by Heidt *et al.*[1], and have produced structures for the O1 (ref. 12), O3 (ref. 11), and O5 (ref. 11) polymers. Now we present results for a polymer from the reference strain (A.T.C.C. 17759) for serogroup O7.

Neutral sugar components of the lipopolysaccharide were glucose, mannose, rhamnose, and aldoheptoses (mainly L-*glycero*-D-*manno*-heptose or its enantiomer, with a smaller proportion of D-*glycero*-D-*manno*-heptose or its enantiomer). Mild acid hydrolysis of the lipopolysaccharide in the presence of sodium dodecyl sulphate[13] apparently did not release lipid A completely: the major polymeric fraction (yield, 24%) eluted from Sephadex G-50 was preceded by phosphorus-containing carbohydrate (16%), assumed to be undegraded lipopolysaccharide (phosphorus being a characteristic component of lipid A, and often of the core oligosaccharide). Incomplete release of lipid A by the method used here has also been observed with the lipopolysaccharide from *P. cepacia* serogroup O3 (ref. 11). In addition to the polymeric fractions, water-soluble products in the O7 hydrolysate included oligomeric material expected to be derived from lipopolysaccharide lacking the O-specific chain. The core oligosaccharide is likely to be the source of the aldoheptoses and also of the rhamnose detected (rhamnose is a minor component of several lipopolysaccharides from *P. cepacia*[14,15], and the core oligosaccharide from strain N.C.T.C. 10661, which is apparently equivalent to A.T.C.C. 17759, contains a rhamnopyranosyl group[16]).

Structural studies were confined to the major polymeric O7 fraction, which consisted mainly of D-glucose (40%) and D-mannose (36%). The ^{1}H-n.m.r. spectrum of the polymer contained three major signals (each 1 H) in the anomeric

0008-6215/90/$ 03.50

TABLE I

^{13}C-N.M.R. DATA[a] FOR THE NATIVE AND THE *O*-DEACETYLATED O7 GLYCAN

Carbon atom	*Native glycan*		*Deacetylated glycan*	
		OAc | 2		
	*→4)-β-Glc*p-*(1→*	*→3)-β-Man*p-*(1→*	*→4)-β-Glc*p-*(1→*	*→3)-β-Man*p-*(1→*
C-1	99.92	99.77	100.87	100.72
C-2	73.36	69.42	73.49	68.91
C-3	75.27	77.48	75.53	79.59
C-4	80.44	66.20	80.79	65.84
C-5	75.15	77.15	74.93	77.05
C-6	60.96	61.68	61.04	61.79
$-OC(O)CH_3$		174.01		
$-OC(O)CH_3$		21.11		

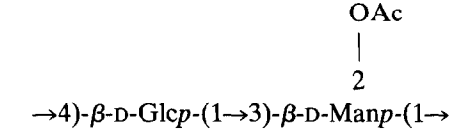
[a]Chemical shifts are given in p.p.m. using 1,4-dioxane (δ 67.40) as the internal reference. Assignments are tentative (based on literature data[9,17]) and may be interchanged for some closely spaced signals.

region at δ 5.59 (apparent doublet, incompletely resolved), 4.88 (unresolved), and 4.49 ($J_{1,2}$ 7.8 Hz), as well as several minor signals (each <0.2 H). Also significant was a methyl singlet at δ 2.14, suggesting the presence of an *O*-acetyl group. Mild alkaline treatment of the polymer caused loss of the signals at δ 5.59 and 2.14, and shifts of the true anomeric signals to δ 4.73 (unresolved) and 4.60 ($J_{1,2}$ 7.8 Hz). These data indicated (*a*) that the repeating unit of the polymer was a disaccharide of glucose and mannose, (*b*) that both sugars were present as β-pyranosyl residues, and (*c*) that mannose was *O*-acetylated at position 2 (inferred from the low *J* values for the signal at δ 5.59).

The ^{13}C-n.m.r. spectra for the native and the *O*-deacetylated polymer confirmed and extended the above inferences on structure. The spectrum for the native polymer contained 14 discrete major signals, including anomeric signals at δ 99.92 ($^1J_{CH}$ 163 Hz) and 99.77 ($^1J_{CH}$ 163 Hz), acetyl signals at δ 174.01 and 21.11, and signals for unsubstituted hydroxymethyl groups at δ 61.68 and 60.96. For the *O*-deacetylated polymer, the anomeric signals (δ 100.87 and 100.72) were at lower field.

Methylation analysis of the polymer gave only one major peak in g.l.c. of the methylated alditol acetates. However, the primary fragment ions obtained in e.i.-m.s. (*m/z* 118, 161, 233, 234, and 277) indicated that the material was a mixture of the derivatives from 3-substituted and 4-substituted hexopyranosyl residues. G.l.c. analysis of reference compounds showed that the unresolved peak corresponded to the derivatives from 3-substituted mannose and 4-substituted glucose. Thus, structure **1** could be assigned to the repeating unit for the O7 polymer. The ^{13}C-n.m.r. data (Table I) were in accord with this structure. In particular, the upfield

shift of the signal for C-2 of mannose, and the downfield shifts for C-1 and C-3, on *O*-deacetylation confirm the location of the *O*-acetyl group.



1

The structure established for the polymer from the O7 reference strain (A.T.C.C. 17759) is identical with that reported[9] for a polymer from strain IMV 4207, and the n.m.r. data are in excellent agreement. Strain IMV 4207 apparently belongs to serogroup A in the classification of Nakamura *et al.*[2], which corresponds to O7 in the scheme of Heidt *et al.*[1]. The results of this study and that by Knirel *et al.*[9] confirm this correspondence; it is interesting to note that a polymer with the repeating unit **1** is also present[9] in a strain (IMV 598/2) that is related to both serotype A (ref. 2) and O2 (ref. 1).

Whereas some O antigens of *P. cepacia* contain unusual sugars, *e.g.*, D-rhamnose[4,6], L-*glycero*-D-*manno*-heptose[6], D-fucose[7], and 2-amino-2-deoxy-L-glucose[12], the O7 polymer is unexceptional in composition. The stoichiometric, regiospecific *O*-acetylation of the polymer isolated after mild acid hydrolysis of the lipopolysaccharide is presumably due, in part, to the absence of a vicinal hydroxyl group.

EXPERIMENTAL

Growth of bacteria, and isolation and fractionation of lipopolysaccharide. — The O7 reference strain[1] was grown as described[18]. The wet cells (109 g) were used to prepare cell walls (2.29 g), and the lipopolysaccharide (237 mg) was extracted from defatted walls as in related studies[11,12]. Mild acid hydrolysis was carried out as described by Caroff *et al.*[13], and the water-soluble products were fractionated on Sephadex G-50.

Identification and determination of neutral sugars. — Samples were hydrolysed with 2M HCl for 2 h at 105°. After neutralisation and deionisation of the hydrolysates, neutral sugars were identified by p.c. using ethyl acetate–pyridine–water (13:5:4) and by g.l.c. of the alditol acetates. D-Glucose was determined enzymically[12] and D-mannose was also determined by the hexokinase method in conjunction with D-glucose 6-phosphate dehydrogenase (EC 1.1.1.49), D-glucose phosphate isomerase (EC 5.3.1.9), and D-mannose phosphate isomerase (EC 5.3.1.8).

Structural methods. — In general, n.m.r. spectra (^1H and ^{13}C) were recorded with a Bruker WH-400 spectrometer (all data cited) for solutions in D_2O. ^1H-n.m.r. spectra were recorded at 70° with sodium 3-trimethylsilylpropanoate as the external

reference. ^{13}C-N.m.r. spectra were recorded at 27° (with complete proton-decoupling or with coupling and the INEPT pulse sequence), using 1,4-dioxane as the internal reference. Methods used for *O*-deacetylation and for methylation analysis were those described[12].

ACKNOWLEDGMENTS

We thank the M.R.C. for a project grant, and the S.E.R.C. for access to the high-field n.m.r. service at the University of Warwick. We also thank the staff at Warwick and our colleagues (Miss L. Galbraith, Mrs. B. Worthington, Dr. D. F. Ewing, and Mr. A. D. Roberts) for technical assistance and instrumental services, and Professor H. Monteil (Strasbourg) for the culture of *P. cepacia*.

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