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Preliminary communication

The structure of *Proteus penneri* strain 14 O-specific polysaccharide containing D- and L-alanine

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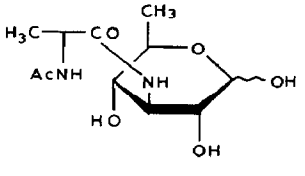
Recently[1], the structure of the O-specific polysaccharide from *Proteus penneri* strain 16 has been established. We now report on the structure of the *P. penneri* strain 14 O-specific polysaccharide (PS).

The PS was obtained on mild hydrolysis (aqueous 1% CH_3COOH, 100°) of the lipopolysaccharide isolated from dry bacterial cells by phenol–water extraction[2], and had $[\alpha]_D$ +9.9° (*c* 2.1, water).

Hydrolysis of the PS (2M CF_3COOH, 120°) yielded galactose, ribose, galacturonic acid, 2-amino-2-deoxyglucose, 3-amino-3,6-dideoxyglucose, and alanine identified by g.l.c.–m.s. as the alditol acetates and with the appropriate help of a sugar and an amino acid analyser. After solvolysis of the PS with anhydrous hydrogen fluoride (20°), the amino components were isolated with their *N*-acyl substituents unaffected, and identified by ^1H- and ^{13}C-n.m.r. spectroscopy as 2-acetamido-2-deoxyglucose, 3-(*N*-acetylalanyl)amino-3,6-dideoxyglucose (**1**), and *N*-(galacturonoyl)alanine (**2**). The structure of **2** was confirmed by the mass spectrum of the corresponding alditol acetate. The D configuration of the monosaccharides was established on the basis of $[\alpha]_D$ values. H.p.l.c. analysis on a chiral phase (C_{18} treated with C_{10}N-L-Hyp) showed that **1** and **2** contained D- and L-alanine, respectively.



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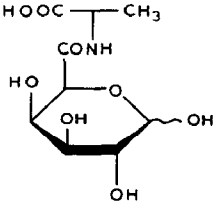

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The ^{1}H- and ^{13}C-n.m.r. spectra of the PS were typical for a regular polymer and, in accord with the results of sugar analysis, showed that the PS has a repeating unit containing five sugar residues, two Ala residues, and two NAc groups.

The ^{1}H-n.m.r. spectrum was assigned with the help of 2D correlated (COSY) and relayed coherence transfer (COSYRCT) spectroscopy, and the ^{13}C-n.m.r. spectrum was interpreted by using shift-correlated heteronuclear ^{13}C–^{1}H (XHCORRD) spectroscopy (Table I). The relatively large vicinal coupling constants (8–10 Hz) found for all of the ring protons of the 6-deoxy sugar, *viz.* **1**, indicated it to be pyranosidic and β-linked. The coupling constants of ring protons of the residues of GalA–Ala (**2**) and Gal were typical for the α-pyranosidic and β-pyranosidic forms, respectively. The β-Rib*f* residue was recognised by the correlation of the signal for H-1 at 5.40 p.p.m. to that for C-1 at 108.7 p.p.m., and the β-Glc*p*NAc residue by the correlations of the signals for H-1 and H-2 at 4.75 and 3.79–3.84 p.p.m. to those for C-1 and C-2 at 102.0 and 55.9 p.p.m., respectively.

TABLE I

^{13}C-N.m.r. data (δ in p.p.m.)

Sugar unit	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
→2)-β-D-Qui*p*3N(Ac-D-Ala)-(1→	104.7	78.3	58.4	75.5	74.4	18.3
→4)-α-D-Gal*p*A(L-Ala)-(1→	99.6	70.3	71.8	81.7	72.5	170.7
→2)-β-D-Rib*f*-(1→	108.7	82.0	71.8	85.0	64.2	
→4)-β-D-Gal*p*-(1→	105.0	72.5	74.6	77.8	76.2	63.1
→3)-β-Glc*p*NAc-(1→	102.0	55.9	84.2	70.7	77.6	62.7

Additional signals: NAc at 23.7 and 24.0 p.p.m. (CH_3), and 175.7 p.p.m. (CO); D-Ala at 18.4 (C-2), 51.8 (C-3), and 178.3 p.p.m. (C-1); L-Ala at 18.5 (C-2), 50.4 (C-3), and 177.1 p.p.m. (C-1).

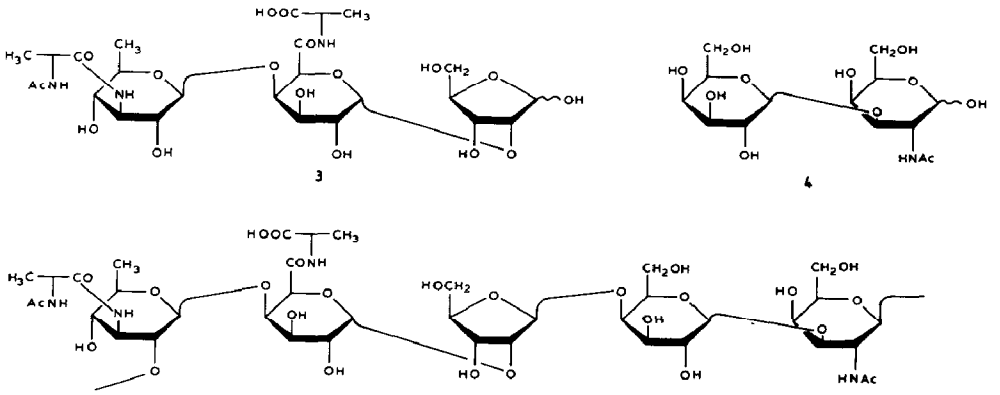
The relatively low-field positions in the ^{13}C-n.m.r. spectrum of the signals for C-2 of residues **1** and Rib, C-3 of the GlcNAc residue, and C-4 of the residues of **2** and Gal, as compared to those in the corresponding free monosaccharides, was due to the α-effects of glycosylation[3] and showed that the PS was linear and substantiated the positions of substitution of the constituent monosaccharides.

On sequential pre-irradiation of H-1 of each of the sugar residues, n.O.e.'s were observed which confirmed the substitution patterns and allowed determination of the sequence of the sugar residues as depected below.

The structure of the PS was confirmed by methylation analysis[4], and by partial hydrolysis (0.1M HCl, 100°) which gave two major oligosaccharides **3** and **4** isolated by gel-permeation chromatography followed by reverse-phase h.p.l.c. Sugar analysis and ^{13}C-n.m.r. spectroscopy established the structures of **3** and **4**, which were in accord with the structure **5** of the PS suggested on the basis of the n.m.r. studies.

The PS contains two amino components that are uncommon for bacterial polysaccharides. One of them, *N*-(D-galacturonoyl)-L-alanine, had been identified[5] as a constituent of the *Proteus mirabilis* O27 O-specific polysaccharide but was isolated for

the first time in this work. As for 3-(*N*-acetyl-D-alanyl)amino-3,6-dideoxy-D-glucose, to our knowledge, it has not been found hitherto in Nature. Its analogue, 3-(*N*-acetyl-L-seryl)amino-3,6-dideoxy-D-glucose, was discovered[6] in the *Escherichia coli* O114 O-specific polysaccharide (structure **6**), which has several other structural similarities with the PS of *P. penneri* (structure **5**).



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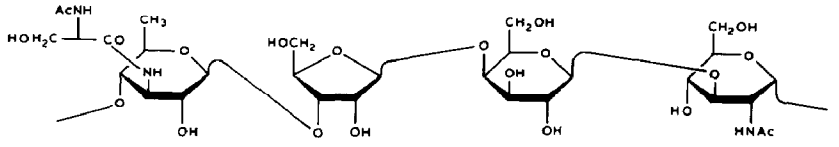
5 (*Proteus penneri* strain 14)



6 (*Escherichia coli* O114)

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