Preliminary communication

Structure of the O-specific polysaccharide of *Proteus penneri* 62 containing 2-acetamido-3-*O*-[(*S*)-1-carboxyethyl]-2-deoxy-D-glucose (*N*-acetylisomuramic acid)

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Recently^{1,2}, the O-specific polysaccharides of two *Proteus penneri* strains 14 and 16 have been structurally elucidated and found to contain N-linked L-alanine, N-acetyl-D-alanyl groups, and (R)-3-hydroxybutyryl groups as non-sugar substituents. We report now the structure of the O-antigen of strain 62 of this new *Proteus* species, which includes a residue of (S)-lactic acid.

The polysaccharide (PS-I) was obtained by mild acid degradation of the lipopolysaccharide, isolated from bacterial cells by the phenol-water procedure³. As judged by the ¹H and ¹³C NMR spectra, PS-I lacked strict regularity, most probably due to the presence of OAc groups in a non-stoichiometric amount ($\delta_{\rm H}$ 2.13, $\delta_{\rm C}$ 21.6).

Treatment of PS-I with 10% aqueous ammonia (60°, 2 h) led to an O-deacety-lated polysaccharide (PS-II), which had a trisaccharide repeating unit; there were signals for three anomeric protons at δ 4.59, 4.69, and 5.40, and carbons at δ 100.5, 103.0, and 103.8. It included two N-acetylated amino sugars [signals for C-2 at δ 55.4 and 57.0, and for two NAc groups: $\delta_{\rm H}$ 2.07 and 2.10; $\delta_{\rm C}$ 23.6 and 23.9 (Me), 175.7 and 175.9 (CO)] and an ether-linked lactic acid [$\delta_{\rm H}$ 1.31 (3 H, d, $J_{2',3'}$ 7 Hz, H-3'), 4.05 (1 H, q, H-2'); $\delta_{\rm C}$ 20.3 (C-3'), 182.9 (C-1')]⁴.

The ¹H NMR spectrum of PS-II was interpreted with the help of sequential, selective spin decoupling, 2D homonuclear shift-correlated spectroscopy (COSY)

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TABLE I 250-MHz 1 H NMR data (δ in ppm, J in Hz)

N-Aceylmuramic acid α 3.72 3.56 3.84 3.84 3.77 4.47 1.44 α 5.24 3.88 3.72 α 3.56 3.84 3.84 3.77 4.47 1.44 α 5.24 3.88 3.72 α 5.24 3.89 5.5 α 5.45 3.91 α 6.45 α 7.77 α 7.77 α 7.79 α 7.71 3.68 3.54 3.54 3.54 3.54 3.54 3.45 3.91 3.74 4.39 1.42 α 7.14 α 7.15 α 7.15 α 7.15 α 7.15 α 7.16 α 7.17 α 7.17 α 7.19 α 7.10 α 7.11 α 7.12 α 7.12 α 7.13 α 7.14 α 7.15	İ	H-1	H-2	H-3	H-4	H-5	H-6a	49-H	H-2′	H-3′	NAc
3.72 3.56 3.84 3.84 3.77 447 447 J_{344} 9.5 $J_{4,5}$ 9.5 $J_{5,6b}$ 5.5 3.91 J_{34} 9.2 $J_{5,6b}$ 5.5 $J_{5,6b}$ 5.5 $J_{5,6b}$ 6.5 $J_{5,6b}$ 12.4 $J_{2,3}$ 7 $J_{6a,6b}$ 12.4 $J_{2,3}$ 7 $J_{6a,6b}$ 12.4 $J_{2,3}$ 7 $J_{6a,6b}$ 12.4 $J_{2,3}$ 7 J_{24} 9.5 $J_{5,6b}$ 5.5 $J_{5,6b}$ 5.5 $J_{5,6b}$ 6.3 3.86 3.86 3.76 4.24 J_{34} 9.5 $J_{4,5}$ 9.5 $J_{5,6b}$ 5.5 $J_{5,6b}$ 5.5 $J_{5,6b}$ 6.3 3.90 3.74 $J_{2,3}$ 7.1 $J_{3,4}$ 9.5 $J_{4,5}$ 9.5 $J_{5,6b}$ 5.5 $J_{5,6a}$ 2.5 $J_{6a,6b}$ 12.5 $J_{2,3}$ 7.1 $J_{3,4}$ 9.5 $J_{4,5}$ 9.5 $J_{5,6b}$ 5.5 $J_{5,6a}$ 2.5 $J_{6a,6b}$ 12.5 $J_{6a,6b}$ 12.5 $J_{2,3}$ 7.1 $J_{3,4}$ 9.5 $J_{4,5}$ 9.5 $J_{5,6b}$ 5.5 $J_{5,6a}$ 2.5 $J_{6a,6b}$ 12. $J_{6a,6b}$ 12. $J_{6a,6b}$ 12. $J_{2,3}$ 7.1 $J_{3,4}$ 9.5 $J_{4,5}$ 9.5 $J_{5,6b}$ 5.5 $J_{5,6a}$ 2.7 $J_{6a,6b}$ 12. $J_{6a,6b}$ 12. $J_{2,3}$ 7.1 $J_{3,4}$ 9.5 $J_{4,5}$ 9.5 $J_{5,6b}$ 5.5 $J_{5,6a}$ 2.7 $J_{6a,6b}$ 12. $J_{6a,6b}$ 12. $J_{2,3}$ 7.1 $J_{3,4}$ 9.5 $J_{4,5}$ 9.5 $J_{5,6b}$ 5.5 $J_{5,6a}$ 2.7 $J_{6a,6b}$ 12. $J_{6a,6b}$ 12. $J_{2,3}$ 7.1 $J_{3,4}$ 9.5 $J_{4,5}$ 9.5 $J_{5,6b}$ 5.5 $J_{5,6a}$ 2.7 $J_{6a,6b}$ 12. J	N-4c	etylmuramic ac	id								
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	α	5.24	3.88	3.72	3.56	3.84	3.84	3.77	4.47	1.44	2.02
3.54 3.54 3.54 3.45 3.91 3.74 4.39 1 3.74 4.39 1 3.74 $\frac{3.54}{J_{5,6b}} 5$ $J_{5,6b} 5$ $J_{5,6b} 5$ $J_{5,6b} 2.5$ $J_{6a,6b} 12.4$ $J_{2',3'} 7$ 3.6 3.86 3.76 4.24 1 3.48 3.57 3.46 3.90 3.74 4.22 1 3.74 3.71 3.91 3.74 3.74 3.91 3.74 3.74 3.91 3.74 3.91 3.74 3.74 3.91 3.74 3.91 3.74 3.91 3.74 3.91 3.74 3.91 3.74 3.91 3.74 3.91 3.74 3.91 3.74 3.91 3.74 3.91 3.74 3.91 3.74 3.91 3.74 3.91 3.74 3.91 3.74 3.91 3.74 3.91 3.74 3.91 3.74 3.91 3.77 3.83 4.05 1 3.55 $J_{5,6b} 5.5 J_{5,6a} 2.5 J_{6a,6b} 12.5 J_{6a,6b} 12.5 J_{5,6a} 2.5 J_{5$		$J_{1,2}$ 3.5		$J_{3.4}$ 9.5	$J_{4.5}$ 9.5	Js. 5.5		J_{6a} 6h 12.4	1,1,7		
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$\begin{array}{cccccccccccccccccccccccccccccccccccc$	α	5.17	4.03	3.67	3.59	3.86	3.86	3.76	4.24	1.36	2.09
3.48 3.57 3.46 3.90 3.74 4.22 4.22 4.24 4.22 4.24 4.22 4.25 4.24 9.5 $I_{3,4}$ 9.5 $I_{5,66}$ 5.5 $I_{5,66}$ 2.5 $I_{6a,6b}$ 12.5 $I_{2',3'}$ 7.1 3.11 $I_{3,4}$ 9.5 $I_{4,5}$ 9.5 $I_{5,6b}$ 5.5 $I_{5,6a}$ 2.5 $I_{6a,6b}$ 12.5 $I_{2',3'}$ 7.1 $I_{3,4}$ 9.5 $I_{4,5}$ 9 $I_{5,6b}$ 5 $I_{5,6a}$ 2 $I_{6a,6b}$ 12 $I_{6a,6b}$ 12 $I_{6a,6b}$ 12 $I_{2,4}$ 9.5 $I_{4,5}$ 9.5 $I_{5,6b}$ 5.5 $I_{5,6a}$ 2 $I_{6a,6b}$ 12 $I_{2,3'}$ 7 $I_{3,4}$ 9.5 $I_{4,5}$ 9.5 $I_{5,6b}$ 5.5 $I_{5,6a}$ 2 $I_{6a,6b}$ 12 $I_{2',3'}$ 7 $I_{3,4}$ 9.5 $I_{4,5}$ 9.5 $I_{5,6b}$ 5.5 $I_{5,6a}$ 2 $I_{6a,6b}$ 12 $I_{2,3'}$ 7 $I_{3,4}$ 2.5 $I_{4,5}$ 4.18 3.88 3.72 3.72		$J_{1.2} 3.7$	$J_{2,3}$ 9.7	$J_{3.4}$ 9.5	$J_{4.5}$ 9.5	J _{5.60} 5.5		Jan 64 12.5	J, 1, 7.1		
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PS-II) yyranose (unit A) 3.74 3.47 3.91 3.74 3.74 3.74 3.74 3.74 $J_{3,4} = J_{4,5} = J_{5,6b} = J_{5,6b} = J_{5,6a} = J_{6a,6b} = J_{6a,6b$		$J_{1.2} 8.9$	$J_{2,3}$ 9.5	$J_{3.4}$ 9.5	$J_{4,5} 9.5$	$J_{5,6b}$ 5.5	$J_{5.6a}$ 2.5	J _{6a,6b} 12.5	$J_{2',3'}$ 7.1		
ranose (unit A) 3.74 3.47 3.91 3.74 3.74 3.74 3.74 3.74 3.77 $J_{3,4}$ 9 $J_{4,5}$ 9 $J_{5,6b}$ 5 $J_{5,6b}$ 2 $J_{6a,6b}$ 12 3.83 4.05 3.43 3.73 3.73 3.72 3.72 3.72 3.74 3.74 3.74 3.74 3.74 3.74 3.74 3.74	O-De	acetylated polys	saccharide (PS-I	(
3.77 3.74 3.47 3.91 3.74 $f_{3.4}$ 3.74 3.91 3.74 $f_{3.4}$ 9 $f_{4.5}$ 9 $f_{5.6b}$ 5 $f_{5.6a}$ 2 $f_{6a.6b}$ 12 $f_{6a.6b}$ 12 3.83 4.05 $f_{3.4}$ 9.5 $f_{4.5}$ 9.5 $f_{5.6b}$ 5.5 $f_{5.6b}$ 5.5 $f_{5.6a}$ 2 $f_{6a.6b}$ 12 $f_{2.3'}$ 7 3.77 4.18 3.88 3.72 3.72 3.72 $f_{2.4}$ 2.5 $f_{4.5}$ 5.4 3.89 3.72 3.72	2-Ace	tamido-2-deoxy	-β-D-glucopyran	nose (unit A)							
$J_{3,4}$ 9 $J_{4,5}$ 9 $J_{5,6b}$ 5 $J_{5,6a}$ 2 $J_{6a,6b}$ 12 $J_{4,5}$ 9 $J_{5,6b}$ 5 $J_{5,6a}$ 2 $J_{6a,6b}$ 12 $J_{3,4}$ 9.5 $J_{4,5}$ 9.5 $J_{5,6b}$ 5.5 $J_{5,6a}$ < 2 $J_{6a,6b}$ 12 $J_{2',3'}$ 7 $J_{3,4}$ 9.7 4.18 3.88 3.72 3.72		4.59	3.64	3.77	3.74	3.47	3.91	3.74			2.07 "
thyl]-2-deoxy- β -D-glucopyranose (unit B) 3.43 3.57 3.55 4.12 3.83 4.05 $J_{3,4}$ 9.5 $J_{4,5}$ 9.5 $J_{5,6b}$ 5.5 $J_{5,6b}$ 5.5 $J_{5,6a}$ <2 $J_{6a,6b}$ 12 $J_{2',3'}$ 7 3.77 4.18 3.88 3.72 3.72 3.72		$J_{1,2}$ 7.5	$J_{2,3}$ 9	$J_{3,4}$ 9	14.5 9	$J_{5,6b}$ 5	$J_{5.6a}$ 2	$J_{6a,6b}$ 12			
3.43 3.57 3.55 4.12 3.83 4.05 $J_{3,4}$ 9.5 $J_{4,5}$ 9.5 $J_{5,6b}$ 5.5 $J_{5,6a}$ <2 $J_{6a,6b}$ 12 $J_{2',3'}$ 7 3.77 4.18 3.88 3.72 3.72 3.72 $J_{3,4}$ 2.5 $J_{4,5}$ <1	2-Ace	tamido-3-O-[(S	ethy	l]-2-deoxy-β-D-g	glucopyranose (unit B)					
$J_{3,4}$ 9.5 $J_{4,5}$ 9.5 $J_{5,6b}$ 5.5 $J_{5,6a}$ <2 $J_{6a,6b}$ 12 $J_{2',3'}$ 7 3.77 4.18 3.88 3.72 3.72 $J_{4,5}$ <1		4.69		3.43	3.57	3.55	4.12	3.83	4.05	1.31	2.10 "
3.77 4.18 3.88 3.72 $J_{3,4}$ 2.5 $J_{4,5} < 1$		$J_{1.2}$ 8.4		$J_{3.4}$ 9.5	$J_{4.5}$ 9.5	J _{5.6b} 5.5	$J_{5,6a} < 2$	$J_{6a,6b}$ 12	J2',3' 7		
3.77 4.18 3.88 3.72 $J_{3,4}$ 2.5 $J_{4,5} < 1$	α-D-C	ialactopyranose									
J ₃₄ 2.5		5.40	3.89	3.77	4.18	3.88	3.72	3.72			
		$J_{1,2}$ 3.6	$J_{2,3} 10$	J _{3,4} 2.5	$J_{4.5} < 1$						

^a Assignment could be interchanged.

TABLE II 75-MHz ¹³C NMR data for PS-II (8 in ppm)

•	C-1 C-2 C-3	C-3	C-4	C-5	ပို	C-1,	C-2,	C-3,	C-4 C-5 C-6 C-1' C-2' C-3' CH ₃ CON CH ₃ CON	CH ₃ CON
-Acetamii 03.0	do-2-deoxy-β- 55.4	2-Acetamido-2-deoxy-β-D-glucopyranose (unit A) 55.4 80.9 72.2	tose (unit A) 72.2	76.9	62.0				23.6 4	175.7 6
-Acetamii 03.8	do-3-O-[(S)-1 57.0	-carboxyethyl 84.4	2-Acetamido-3-O-[(S)-1-carboxyethyl]-2-deoxy-β-D-glucopyranose (unit B) 103.8 57.0 84.4 69.7	-glucopyranos 75.5	e (unit B) 69.7	182.9	80.5	20.3	23.9 a	175.9 b
:-D-Galac. 00.5	α-D-Galactopyranose (unit C) 100.5 68.8 80.9	mit C) 80.9	70.0	72.0 62.0	62.0					

a.b Assignment could be interchanged.

and COSY with one- and two-step relayed coherence transfer (COSYRCT) (Table I), and then the ¹³C NMR spectrum of PS-II was assigned by using 2D heteronuclear ¹³C-¹H shift-correlated spectroscopy (Table II).

The ${}^3J_{\rm H,H}$ coupling constants indicated that both amino sugars were derivatives of 2-amino-2-deoxy- β -glucose (units **A** and **B**), and that the third sugar was α -galactose (unit **C**). Pre-irradiation of H-2 of the lactic acid residue resulted in a marked NOE on H-3 of unit **B**, which proved this unit to be a residue of N-acetylmuramic acid or its stereoisomer. This conclusion accorded with a low-field position at δ 84.4 of the signal for C-3 of unit **B**.

Solvolysis of PS-II with anhydrous HF⁵ (20°, 3 h) led to D-galactose and 2-acetamido-2-deoxy-D-glucose, which were conventionally identified, and an N-acetylated acidic amino sugar, isolated by anion-exchange HPLC on TSK DEAE-3SW in 2% acetic acid. The ¹H NMR spectra of this monosaccharide and N-acetylmuramic acid were different (Table I), and the corresponding amino sugars, obtained by acid hydrolysis, had quite different retention times in amino acid analysis ($T_{\rm Glu}$ 1.33 and 1.00, respectively). A small negative [α]_D value (-6°, H₂O) allowed identification of the isolated sugar as 2-acetamido-3-O-[(S)-1-carboxyethyl]-2-deoxy-D-glucose (1), i.e., so-called N-acetylisomuramic acid (cf. the published values +48.3° and -28.7° for N-acetylmuramic and N-acetylisomuramic acid, respectively).

Linkage and sequence analysis of PS-II was carried out by using NOE spectroscopy⁷ with sequential pre-irradiation of H-1 of each of the sugar units. The NOE contacts thus observed (H-1 A/H-6a,6b B; H-1 B/H-3 C; H-1 C/H-3 A) showed that PS-II was linear with the sequence A-B-C, units A and C were 3-substituted, and unit B was 6-substituted. The substitution pattern was confirmed by low-field displacements of the signals for C-3 of units A and C to 80.9 ppm and C-6 of unit B to 69.7 ppm, which were caused by the α -effects of glycosylation⁸. The chemical shift (δ 103.8) for C-1 of unit B proved the sugar residue in this unit to have the same absolute configuration as galactose, i.e., the D configuration (a chemical shift near δ 96 would be expected⁹ in the case of different absolute configurations of β -(1 \rightarrow 3)-linked units B and C). This conclusion confirmed identification of unit B as the residue of 1.

The 13 C NMR spectrum of PS-I exhibited two series of the signals in the ratio $\sim 2:1$, which belonged to O-acetylated and non-acetylated repeating units, respectively. Comparison of the former series with the spectrum of PS-II showed marked

displacements of the signals for C-6 and C-5 of unit A from δ 62.0 and 76.9 to 64.5 and 74.5, which corresponded to the α - and β -effects, respectively, of *O*-acetylation¹⁰. Hence, unit A carried the OAc group at position 6 in \sim 70% of the repeating units of PS-I.

On the basis of the data obtained, one can conclude that the O-specific polysaccharide of *P. penneri* strain 62 has the following structure:

where Sug is the residue of 1.

To the best of our knowledge, N-acetylisomuramic acid 1 has not been found hitherto in Nature. N-Acetylmuramic acid, a well-known component of the bacterial cell-wall peptidoglycan, has been described 11 also as a component of the O-antigen of Yersinia ruckerii. The (R)- and (S)-1-carboxyethyl ethers of other sugars occur in a number of bacterial polysaccharides 12, including Proteus vulgaris $O25^{13}$.

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