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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 procedure[3]. As judged by the ^1H and ^{13}C NMR spectra, PS-I lacked strict regularity, most probably due to the presence of OAc groups in a non-stoichiometric amount (δ_H 2.13, δ_C 21.6).

Treatment of PS-I with 10% aqueous ammonia (60°, 2 h) led to an *O*-deacetylated 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: δ_H 2.07 and 2.10; δ_C 23.6 and 23.9 (Me), 175.7 and 175.9 (CO)] and an ether-linked lactic acid [δ_H 1.31 (3 H, d, $J_{2',3'}$ 7 Hz, H-3'), 4.05 (1 H, q, H-2'); δ_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)

	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	H-2′	H-3′	NAc
N-*Acetylmuramic acid*										
α	5.24	3.88	3.72	3.56	3.84	3.84	3.77	4.47	1.44	2.02
	$J_{1,2}$ 3.5	$J_{2,3}$ 10.3	$J_{3,4}$ 9.5	$J_{4,5}$ 9.5	$J_{5,6b}$ 5.5		$J_{6a,6b}$ 12.4	$J_{2',3'}$ 7		
β	4.71	3.68	3.54	3.54	3.45	3.91	3.74	4.39	1.42	2.00
	$J_{1,2}$ 8.0	$J_{2,3}$ 10		$J_{4,5}$ 9.5	$J_{5,6b}$ 5	$J_{5,6a}$ 2.5	$J_{6a,6b}$ 12.4	$J_{2',3'}$ 7		
N-*Acetylisomuramic acid* (**1**)										
α	5.17	4.03	3.67	3.59	3.86	3.86	3.76	4.24	1.36	2.09
	$J_{1,2}$ 3.7	$J_{2,3}$ 9.7	$J_{3,4}$ 9.5	$J_{4,5}$ 9.5	$J_{5,6b}$ 5.5		$J_{6a,6b}$ 12.5	$J_{2',3'}$ 7.1		
β	4.75	3.76	3.48	3.57	3.46	3.90	3.74	4.22	1.36	2.09
	$J_{1,2}$ 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		
O-*Deacetylated polysaccharide* (PS-II)										
*2-Acetamido-2-deoxy-β-*D*-glucopyranose (unit* **A**)										
	4.59	3.64	3.77	3.74	3.47	3.91	3.74			2.07 [a]
	$J_{1,2}$ 7.5	$J_{2,3}$ 9	$J_{3,4}$ 9	$J_{4,5}$ 9	$J_{5,6b}$ 5	$J_{5,6a}$ 2	$J_{6a,6b}$ 12			
*2-Acetamido-3-O-[(S)-1-carboxyethyl]-2-deoxy-β-*D*-glucopyranose (unit* **B**)										
	4.69	3.79	3.43	3.57	3.55	4.12	3.83	4.05	1.31	2.10 [a]
	$J_{1,2}$ 8.4	$J_{2,3}$ 9.2	$J_{3,4}$ 9.5	$J_{4,5}$ 9.5	$J_{5,6b}$ 5.5	$J_{5,6a} < 2$	$J_{6a,6b}$ 12	$J_{2',3'}$ 7		
*α-*D*-Galactopyranose (unit* **C**)										
	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 ^{13}C NMR data for PS-II (δ in ppm)

C-1	C-2	C-3	C-4	C-5	C-6	C-1′	C-2′	C-3′	CH_3CON	CH_3CON
2-Acetamido-2-deoxy-β-D-glucopyranose (unit **A**)										
103.0	55.4	80.9	72.2	76.9	62.0				23.6 [a]	175.7 [b]
2-Acetamido-3-O-[(S)-1-carboxyethyl]-2-deoxy-β-D-glucopyranose (unit **B**)										
103.8	57.0	84.4	70.0	75.5	69.7	182.9	80.5	20.3	23.9 [a]	175.9 [b]
α-D-Galactopyranose (unit **C**)										
100.5	68.8	80.9	70.0	72.0	62.0					

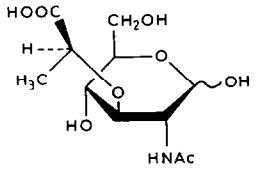
[a,b] Assignment could be interchanged.

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

The $^{3}J_{H,H}$ 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 HF[5] (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 ^{1}H 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_{Glu} 1.33 and 1.00, respectively). A small negative $[\alpha]_D$ value (−6°, H_2O) 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[6] 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 spectroscopy[7] 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 glycosylation[8]. 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 expected[9] in the case of different absolute configurations of β-(1 → 3)-linked units **B** and **C**). This conclusion confirmed identification of unit **B** as the residue of **1**.

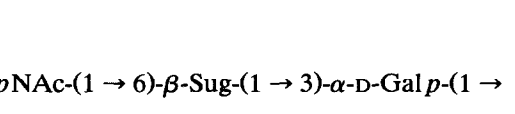


1

The ^{13}C NMR spectrum of PS-I exhibited two series of the signals in the ratio ~ 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*-acetylation[10]. Hence, unit **A** carried the OAc group at position 6 in ~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:



PS-I

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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