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Preliminary communication

2-Acetamido-4-*O*-[(*S*)-1-carboxyethyl]-2-deoxy-D-glucose: a new natural isomer of *N*-acetylmuramic acid from the O-specific polysaccharide of *Proteus penneri* 35

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Recently [1,2], 2-acetamido-3-*O*-[(*R*)- and (*S*)-1-carboxyethyl]-2-deoxy-D-glucoses (*N*-acetylmuramic acid and *N*-acetylisomuramic acid) have been identified as constituents of the O-specific polysaccharides of *Yersinia ruckerii* II and *Proteus penneri* 62, respectively. We report now the isolation and identification of 2-acetamido-4-*O*-[(*S*)-1-carboxyethyl]-2-deoxy-D-glucose from the O-specific polysaccharide of *Proteus penneri* 35.

The polysaccharide was obtained by mild acid degradation (2% CH_3COOH, 100°C) of the lipopolysaccharide isolated from dry bacterial cells by the phenol–water procedure [3]. Its ^{13}C NMR spectrum showed the presence of OAc groups in a nonstoichiometric amount (δ_C 21.5), which were removed by treatment with aqueous 10% ammonia (60°C).

Conventional sugar analysis of the *O*-deacetylated polysaccharide revealed rhamnose, glucose, galactose, and 2-amino-2-deoxyglucose in the ratios ~2:1:1:1. As judged by the ^1H and ^{13}C NMR spectra, the polysaccharide has a hexasaccharide repeating unit (there were signals for six anomeric protons in the region 4.53–5.60 ppm and carbons in the region 99.6–103.6 ppm). It contains two 6-deoxyhexoses [signals for H-6 at δ 1.25 and 1.32 (each 3 H, d, $J_{5,6}$ 6 Hz) and C-6 at δ 18.0 (2 C)], two *N*-acetylated amino sugars [signals for carbons bearing nitrogen at δ 55.9 and 56.5, and for two NAc groups: δ_H 2.02 and 2.06; δ_c 23.7 and

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Table 1
300-MHz ^{1}H NMR data (δ in ppm, J in Hz) [a]

	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	H-2′	H-3′	NAc
2-Acetamido-4-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-D-glucopyranose										
α	5.18	3.86	3.87	3.47	3.86	3.77	3.86	4.38	1.35	2.04
	$J_{1,2}$ 3.1		$J_{3,4}$ 9	$J_{4,5}$ 9	$J_{5,6a}$ 4	$J_{6a,6b}$ 12		$J_{2',3'}$ 7		
β	4.68	3.66	3.66	3.42	3.51	3.78	3.88	4.39	1.36	2.04
	$J_{1,2}$ 8		$J_{3,4}$ 9	$J_{4,5}$ 9	$J_{5,6a}$ 4	$J_{5,6b}$ 4	$J_{6a,6b}$ 12	$J_{2',3'}$ 7		
2-Acetamido-4-*O*-[(*S*)-1-carboxyethyl]-2-deoxy-D-glucopyranose (**1**)										
α	5.17	3.84	3.90	3.42	3.89	3.79	3.88	4.07	1.38	2.04
	$J_{1,2}$ 3.1		$J_{3,4}$ 9	$J_{4,5}$ 9	$J_{5,6a}$ 5		$J_{6a,6b}$ 12	$J_{2',3'}$ 7		
β	4.70	3.68	3.68	3.40	3.50	3.75	3.89	4.07	1.37	2.04
	$J_{1,2}$ 8		$J_{3,4}$ 9	$J_{4,5}$ 9	$J_{5,6a}$ 5	$J_{5,6b}$ 3	$J_{6a,6b}$ 12	$J_{2',3'}$ 7		

[a] The spectra of the NH_4-salts were run in D_2O at 30°C.

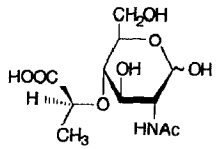
Table 2
75-MHz ^{13}C NMR data (δ in ppm) [a]

	C-1	C-2	C-3	C-4	C-5	C-6	C-1′	C-2′	C-3′	CH_3CON	CH_3CON
2-Acetamido-4-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-D-glucopyranose											
α	91.8	55.4	71.6	78.5	72.2	62.1	182.7	79.2	20.0	23.1	175.6
β	96.2	58.0	75.4	78.3	76.0	62.1	182.7	79.2	20.0	23.4	175.9
2-Acetamido-4-*O*-[(*S*)-1-carboxyethyl]-2-deoxy-D-glucopyranose (**1**)											
α	91.7	55.4	70.8	80.1	71.9	61.2	182.9	79.5	20.1	23.1	175.5
β	96.0	57.7	74.1	79.8	76.5	61.3	182.9	79.5	20.1	23.4	175.8

[a] The spectra of the NH_4-salts were run in D_2O at 30°C.

24.1 (Me), 175.4 and 175.7 (CO)], and an ether-linked lactic acid {δ_H 1.30 (3 H, d, $J_{2',3'}$, 7 Hz, H-3′) and 4.42 (1 H, q, H-2′); δ_C 18.8 (C-3′) and 182.3 (C-1′); cf. data in the literature [2,4]}.

Solvolysis of the *O*-deacetylated polysaccharide with anhydrous HF (20°C) followed by anion-exchange HPLC on TSK DEAE-3SW in 2% acetic acid resulted in isolation of an *N*-acetylated acidic amino sugar (**1**). The ^{1}H and ^{13}C NMR chemical shifts (Tables 1 and 2, respectively) and the $^3J_{H,H}$ coupling constant values (Table 1) showed that **1** is 2-acetamido-2-deoxyglucose etherified by lactic acid. Pre-irradiation of H-2′ of the lactic acid residue at δ 4.07 caused a strong nuclear Overhauser effect on the signals at δ 3.40 (H-4β) and 3.42 (H-4α), thus indicating the substitution of GlcNAc at position 4.



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Authentic 2-acetamido-4-*O*-[(*R*)- and -(*S*)-1-carboxyethyl]-2-deoxy-D-glucoses were synthesized by alkylation of benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside [5] with (*S*)- and (*R*)-2-chloropropionic acid, respectively, followed by hydrogenolysis over Pd–C as described [5]. The natural compound **1** was found to be indistinguishable from the synthetic (*S*)-isomer and different from the (*R*)-isomer by the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2, respectively). The most significant differences between the chemical shifts for the (*R*)- and (*S*)-isomers were observed for H-2′ (δ 4.38–4.39 and 4.07, respectively) and for C-3,4,6 (Table 2). Comparison of the specific optical rotation values of **1** and the synthetic (*S*)-isomer {$[\alpha]_D^{28}$ +8.0° and +8.6° (H_2O), respectively} showed that they have the same absolute configuration {cf. $[\alpha]_D^{28}$ +41.7° (H_2O) for the (*R*)-isomer}.

Therefore, the acidic amino sugar isolated from the O-specific polysaccharide of *P. penneri* 35 is 2-acetamido-4-*O*-[(*S*)-1-carboxyethyl]-2-deoxy-D-glucose (**1**). To the best of our knowledge, this isomer of *N*-acetylmuramic acid has not hitherto been found in Nature.

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