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Note

Identification of N^{ε}-[(*R*)-1-carboxyethyl]-L-lysine in, and the complete structure of, the repeating unit of the O-specific polysaccharide of *Providencia alcalifaciens* O23

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Abstract

N^{ε}-[(*R*)-1-Carboxyethyl]-L-lysine was released by acid hydrolysis from the O-specific polysaccharide of *Providencia alcalifaciens* O23 and identified by ^{1}H and ^{13}C NMR spectroscopy, GLC-MS after conversion to a di-*N*-acetylated dimethyl ester, and by comparison with the authentic sample. Solvolysis of the polysaccharide with anhydrous HF resulted in an amide of D-glucuronic acid with N^{ε}-[(*R*)-1-carboxyethyl]-L-lysine. These and published data allowed the determination of the full structure of the repeating unit of the O-specific polysaccharide.

Keywords: *Providencia alcalifaciens*; O-specific polysaccharide; O-antigen; N^{ε}-[(*R*)-1-Carboxyethyl]-L-lysine; D-Glucuronic acid amide

Providencia is a genus within the family Enterobacteriaceae. On the basis of somatic antigens (lipopolysaccharides) two species, *P. alcalifaciens* and *P. stuarti*, were classified into 62 O-serogroups [1]. *Providencia* is among the least studied enterobacteria with respect to the lipopolysaccharide structure. Recently, we have found an amide of D-glucuronic acid with N^{ε}-(1-carboxyethyl)lysine in the O-specific polysaccharide chain (O-antigen) of the *P. alcalifaciens* O23 lipopolysaccharide [2–4]. The structure of the polysaccharide was established by 2D NMR spectroscopy and selective degradations (partial acid hydrolysis and solvolysis with anhydrous HF) [3,4], but the configuration of the unusual amino acid remained unknown. Now, we report on the identification of this component, including the determination of the absolute configuration.

The O-specific polysaccharide was isolated as described [4] and hydrolyzed with 2 M CF_3COOH (121 °C, 2 h) to give D-Glc, D-Gal, D-GalN and D-GlcA as well as a neutral amino acid **1** which was isolated by preparative PC using the solvent system 5:5:1:3 ethyl acetate–pyridine–acetic acid–water.

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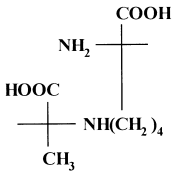
The ^{1}H NMR spectrum of **1** revealed spin systems for lysine and alanine (Table 1). Correspondingly, the ^{13}C NMR spectrum of **1** (Table 1) contained signals for both amino acids but those for C-6 of lysine and C-2′ of alanine were shifted significantly downfield to δ 46.97 and 59.05, as compared with their positions at δ 40.6 and 51.6 in the spectra of the corresponding free amino acids.

These data suggested that **1** is N^{ε}-(1-carboxyethyl)lysine, which was confirmed by GLC–MS analysis of a di-*N*-acetylated dimethyl ester **2** derived from **1**. CIMS revealed for **2** the expected molecular mass of 330 a.m.u. The EI mass spectrum of **2** showed peaks at *m*/*z* 330 (M), 298 (M–MeOH), 287 (M–Ac), 271 (M–$COOCH_3$), 229 (M–$COOCH_3$–CH_2CO), and 211 (M–$COOCH_3$–HOAc).

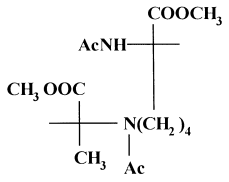
A positive optical rotation value for **1**, $[\alpha]_D + 4.9°$ (*c* 0.5, water), showed that the lysine residue has the L configuration {compare published data [5]: $[\alpha]_D + 9.7°$ and $+11.6°$ (water) for N^{ε}-[(*R*)-1-carboxyethyl]-L-lysine and N^{ε}-[(*S*)-1-carboxyethyl]-L-lysine, respectively}. In order to determine the configuration of the 1-carboxyethyl group, both stereoisomers of N^{ε}-(1-carboxyethyl)-L-lysine were synthesized by condensation of N^{α}-carbobenzoxy-L-lysine with (*S*)- and (*R*)-2-bromopropionic acid followed by deprotection essentially as described [5].

The synthetic diastereomers and the natural amino acid **1** were converted into ammonium salts by absorption on Dowex 50×4 (H^+ form) resin followed by elution with aq 5% ammonia, and then studied by ^{13}C NMR spectroscopy (for reference data, see [6]). The spectrum of a mixture of **1** and N^{ε}-[(*R*)-1-carboxyethyl]-L-lysine and the spectra of the individual compounds were indistinguishable, while two series of signals were present in the spectrum of a mixture of **1** and N^{ε}-[(*S*)-1-carboxyethyl]-L-lysine, the most marked difference being observed for the C-4 chemical shifts (Table 1)[1]. Therefore, the amino acid released from the O-specific polysaccharide of *P. alcalifaciens* O23 is N^{ε}-[(*R*)-1-carboxyethyl]-L-lysine.

Cleavage of the polysaccharide with anhydrous HF (20 °C, 2 h) gave an amide **3** isolated by GPC on TSK HW-40 in water. The ^{1}H and ^{13}C NMR spectra of **3** contained signals for α-Glc*p*A, β-Glc*p*A and N^{ε}-(1-carboxyethyl)lysine. The signal for H-2 of the lysine residue was shifted downfield to δ 4.4, as compared with its position at δ 3.78 in the spectrum of **1**, thus indicating acylation at N-2. Accordingly, C-6 of GlcA resonated at δ 170.0 that is characteristic for hexuronamides (e.g., ref 7). The structure of **3** was finally confirmed by GLC–MS analysis of a gulonamide derivative **4** derived from **3**. CIMS proved for **4** the molecular mass of 676 a.m.u., and EIMS revealed the same fragmentation in the amino acid moiety as in **2** with no significant fragmentation in the gulonic acid residue.



1



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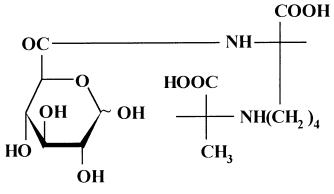

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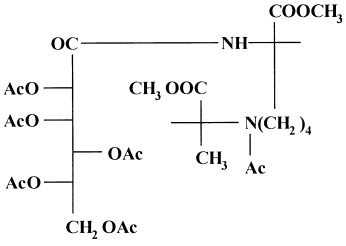

4

Table 1
500-MHz ^{1}H and 125-MHz ^{13}C NMR data (δ, ppm). Spectra were run for solutions of NH_4-salts in D_2O at 20 °C, chemical shifts are referred to acetone (δ_H 2.225, δ_C 31.45)

Proton							
H-2	H-3	H-4	H-5	H-6	H-2′	H-3′	
Amino acid **1**							
3.78	1.94	1.52	1.80	3.09	3.70	1.52	
Carbon							
C-1	C-2	C-3	C-4	C-5	C-6	C-1′	C-2′	C-3′
N^{ε}-[(*R*)-1-Carboxyethyl]-L-lysine and amino acid **1**								
176.15[a]	55.70	31.11	22.77	26.64	46.97	175.82[a]	59.05	16.28
N^{ε}-[(*S*)-1-Carboxyethyl]-L-lysine								
176.15[a]	55.70	31.14	22.82	26.67	46.99	175.82[a]	59.08	16.28

[a] Assignment could be interchanged.

[1] We found that the assignment of the C-3 and C-5 signals previously reported for these compounds [6] was erroneously intercharged.

Therefore, the polysaccharide studied contains N^{ε}-[(*R*)-1-carboxyethyl]-N^{α}-(D-glucuronoyl)-L-lysine (D-GlcA6AlaLys). Taking into account the structure of the carbohydrate backbone of the polysaccharide established earlier by 2D NMR spectroscopy and chemical methods [4], it was concluded that the repeating unit of the O-antigen of *P. alcalifaciens* O23 has the following structure:

$$\rightarrow 6)\text{-}\beta\text{-D-Gal}p\text{-}(1\rightarrow 6)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow 3)\text{-}$$
$$\beta\text{-D-Gal}pNAc\text{-}(1\rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{A6AlaLys-}(1\rightarrow$$

This is the first bacterial polysaccharide reported to contain N^{ε}-[(*R*)-1-carboxyethyl]-L-lysine. A diastereomeric amino acid, N^{ε}-[(*S*)-1-carboxyethyl]-L-lysine, has been found to be produced by *Streptococcus lactis* K1 during growth in an arginine-deficient medium and its biosynthesis suggested to proceed via reductive condensation of lysine with pyruvic acid [6]. Recently, an amide of D-galacturonic acid with N^{ε}-(1-carboxyethyl)lysine of unknown configuration has been reported as a component of the O-specific polysaccharide of *Proteus mirabilis* O13 [8].

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