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Note

Isolation using triflic acid solvolysis and identification of N^{ε}-[(*R*)-1-carboxyethyl]-N^{α}-(D-galacturonoyl)-L-lysine as a component of the O-specific polysaccharide of *Proteus mirabilis* O13

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Abstract

An amino acid was released from the O-specific polysaccharide of *Proteus mirabilis* O13 by acid hydrolysis and identified as N^{ε}-[(*R*)-1-carboxyethyl]-L-lysine by comparison with the authentic sample. An amide of this amino acid with D-galacturonic acid was isolated from the polysaccharide by solvolysis with anhydrous trifluoromethanesulfonic (triflic) acid and characterised by ^{1}H and ^{13}C NMR spectroscopy. These and published data enabled determination of the full structure of the repeating unit of the polysaccharide.

Keywords: *Proteus mirabilis*; O-specific polysaccharide; Structure; N^{ε}-[(*R*)-1-Carboxyethyl]-L-lysine; D-Galacturonic acid amide; Triflic acid solvolysis

Proteus bacteria are human opportunistic pathogens causing common urinary tract infections, which can lead to severe complications such as acute or chronic pyelonephritis and formation of bladder and kidney stones. Recently, structures of O-specific polysaccharides of a number of *Proteus* O-serogroups have been elucidated, and most polysaccharides were found to contain acidic or both acidic and basic components, such as uronic acids, their amides with amino acids, phosphate and phosphate-linked amino components [1]. In particular, the structure of the O-specific polysaccharide of *Proteus mirabilis* O13, which is one of the most common *Proteus* isolates from the clinical material [2], has been established [3]. This polysaccharide has been found to contain N^{ε}-(1-carboxyethyl)-lysine (alaninolysine) [3], but the configuration and the exact location of this unusual amino acid have not been determined. Now, we report on the full identification of this component and present direct evidence of its linkage to galacturonic acid.

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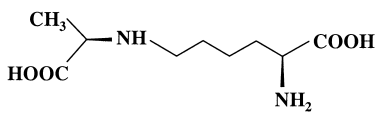
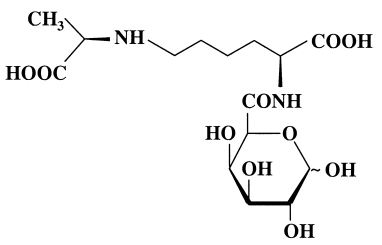
Acid hydrolysis of the O-specific polysaccharide released an amino acid **1** which was separated from monosaccharides by GPC on TSK HW-40. The ^{1}H and ^{13}C NMR spectra of **1** showed signals for lysine and alanine, but the signals for C-6 of lysine and C-2 of alanine were shifted downfield to δ 47.1 and 58.9, as compared with their positions at δ 40.6 and 51.6, respectively, in the corresponding free amino acids. Hence, **1** is N^{ε}-(1-carboxyethyl)lysine. In cation-exchange chromatography, **1** had the same retention time as the authentic sample, and the ^{1}H and ^{13}C NMR chemical shift data of **1** were consistent with published data [4,5].

A positive optical rotation value of **1**, $[\alpha]_D$ + 6.5° (*c* 0.4, water), showed that the lysine residue has the L configuration (compare published data [6]: $[\alpha]_D$ + 9.7 and + 11.6° (water) for N^{ε}-[(*R*)-1-carboxyethyl]-L-lysine and N^{ε}-[(*S*)-1-carboxyethyl]-L-lysine, respectively). The ^{13}C NMR spectrum of a mixture of **1** and the authentic sample of N^{ε}-[(*R*)-1-carboxyethyl]-L-lysine was indistinguishable from the spectra of the individual compounds, whereas two series of signals were present in the spectrum of a mixture of **1** and N^{ε}-[(*S*)-1-carboxyethyl]-L-lysine, the highest difference between the spectra of the isomers being observed for C-4 (0.05 ppm; compare published data [5]). Therefore, **1** is N^{ε}-[(*R*)-1-carboxyethyl]-L-lysine.

Solvolysis of the polysaccharide with anhydrous trifluoromethanesulfonic (triflic) acid was used to isolate a monosaccharide bearing the amino acid. This new reagent in the structural analysis of carbohydrates has been earlier employed for selective cleavage of bacterial polysaccharides [7,8]. Solvolysis resulted in an amide **2** of D-galacturonic acid with amino acid **1**.



1



2

These and published data [7,8] showed that triflic acid cleaves glycosidic linkages, but not amidic linkages, and is thus a useful tool for the isolation of amides of uronic acids and amino sugars with their *N*-acyl substituents. Compared with anhydrous hydrogen fluoride, which is also applicable for this purpose [9], triflic acid cleaves glycosidic linkages more efficiently [7] and does not require special equipment to perform solvolysis.

The ^{1}H and ^{13}C NMR spectra of **2** (Table 1) showed signals for α-GalA, β-GalA and N^{ε}-(1-carboxyethyl)lysine. The signal for H-2 of

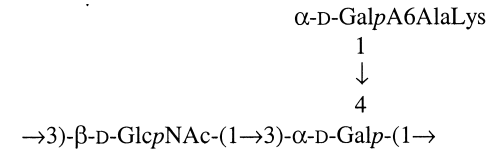
Table 1
500-MHz ^{1}H and 125-MHz ^{13}C NMR data of amide **2** (δ, ppm)

	H-1	H-2	H-3	H-4	H-5	H-6	H-2′	H-3′
α-D-GalA	5.39	3.84	3.93	4.29	4.58			
β-D-GalA	4.68	3.54	3.73	4.23	4.27			
AlaLys		4.41	1.79 [a]	1.47	1.72	3.04	3.70	1.49
	C-1	C-2	C-3	C-4	C-5	C-6	C-2′	C-3′
α-D-GalA	93.7	69.2	70.2	71.0	71.9	172.3		
β-D-GalA	97.6	72.6	73.7	70.4	76.0	172.3		
AlaLys		54.1 [b]	31.8 [c]	23.3	26.4	47.1	58.8	16.1

[a] H-3a; H-3b at δ 1.93.
[b] In α-series; δ 54.2 in β-series.
[c] In α-series; δ 31.7 in β-series.

the lysine residue was shifted downfield to δ 4.4, as compared with its position near δ 3.8 in the free amino acid [5], thus indicating acylation at N-2. Accordingly, C-6 of GalA resonated at δ 172.3 that is characteristic for hexuronamides (e.g., compare $\delta_{C\text{-}6}$ 171.5 in an amide of GalA with threonine [10]). These data are similar to those of an amide of GlcA with N^{ε}-[(*R*)-1-carboxyethyl]-L-lysine [5] and show that **2** is N^{ε}-[(*R*)-1-carboxyethyl]-N^{α}-(D-galacturonoyl)-L-lysine (D-GalA6AlaLys).

Taking into account these and published data [3], it was concluded that the O-specific polysaccharide of *P. mirabilis* O13 has the following structure:



No amide of D-galacturonic acid with N^{ε}-[(*R*)-1-carboxyethyl]-L-lysine has been hitherto found in nature, whereas an amide of the same amino acid with D-glucuronic acid has been previously reported as a component of the O-specific polysaccharide of *Providencia alcalifaciens* O23 [5].

1. 1. Experimental

Bacterial strain, isolation and degradation of lipopolysaccharide.—*P. mirabilis* O13, strain PrK 26/57, from the Czech National Collection of Type Cultures (Institute of Epidemiology and Microbiology, Prague) was cultivated as described [3]. Lipopolysaccharide was obtained from dried bacterial cells by hot phenol/water extraction [11] and degraded with aq 1% HOAc at 100 °C to give a high-molecular-mass O-specific polysaccharide isolated by GPC on Sephadex G-50 (S).

Acid hydrolysis and identification of **1**.—The polysaccharide (33 mg) was hydrolysed with 2 M CF_3COOH (120 °C, 2 h). The hydrolysate was fractionated by GPC on TSK HW-40 (S) to give amino acid **1** (4.5 mg), which was converted into ammonium salt using a column (3 × 1 cm) of an IRA-120 (H^+-form) resin and elution with aq 5% ammonia. **1** was identified by cation-exchange chromatography, ^1H and ^{13}C NMR spectroscopy and a specific optical rotation measured on a Jasco DIP-360 polarimeter (Japan) at 20 °C. Authentic samples of N^{ε}-[(*R*)-1-carboxyethyl]-L-lysine and N^{ε}-[(*S*)-1-carboxyethyl]-L-lysine were synthesised as previously described [5,6].

Triflic acid solvolysis.—The polysaccharide (20 mg) was treated with anhyd CF_3SO_3H (0.7 ml) at −4 °C for 2 h. After neutralisation with aq 25% ammonia at 4 °C and evaporation, the reaction products were fractionated by GPC on TSK HW-40 (S) to give amide **2** (3.6 mg).

Chromatography.—GPC was carried out on a column (56 × 2.6 cm) of Sephadex G-50 (S) (Pharmacia, Sweden) in 0.05 M pyridinium acetate buffer (pH 4.5) or a column (90 × 2.5 cm) of TSK HW-40 (S) (E. Merck, Germany) in deionised water and was monitored with a Knauer differential refractometer (Germany). Cation-exchange chromatography was performed on a Biotronik LC-2000 amino acid analyser (Germany), using a column (0.4 × 22 cm) of an Ostion LG AN B resin and 0.35 M sodium citrate buffer (pH 5.28) at 80 °C.

NMR spectroscopy.—NMR spectra were recorded with a Bruker DRX-500 spectrometer (Germany) for solutions in D_2O at 57 and 27 °C for compounds **1** and **2**, respectively, using internal acetone (δ_H 2.225, δ_C 31.45) as reference. Standard Bruker software (XWIN-NMR 1.2) was used to acquire and process the NMR data. Assignment of the ^1H and ^{13}C NMR spectra of **2** was performed using 2D COSY and H-detected ^1H,^{13}C HMQC experiments, respectively.

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References

[1] Y.A. Knirel, W. Kaca, A. Rozalski, Z. Sidorczyk, *Pol. J. Chem.*, 73 (1999) 895–907.
[2] P. Larsson, *Methods Microbiol.*, 14 (1984) 187–214.

[3] A.S. Shashkov, F.V. Toukach, S.N. Senchenkova, A. Ziolkowski, N.A. Paramonov, W. Kaca, Y.A. Knirel, N.K. Kochetkov, *Biochemistry* (*Moscow*), 62 (1997) 509–513.
[4] J. Thompson, S.P.F. Miller, *J. Biol. Chem.*, 263 (1988) 2064–2069.
[5] N.A. Kocharova, E.V. Vinogradov, S.A. Borisova, A.S. Shashkov, Y.A. Knirel, *Carbohydr. Res.*, 309 (1998) 131–133.
[6] M. Fujioka, M. Tanaka, *Eur. J. Biochem.*, 90 (1978) 297–300.
[7] A.V. Perepelov, S.N. Senchenkova, A.S. Shashkov, N.A. Komandrova, S.V. Tomshich, L.S. Shevchenko, Y.A. Knirel, N.K. Kochetkov, *J. Chem. Soc., Perkin Trans. 1*, in press.
[8] A.V. Perepelov, S.N. Senchenkova, A. Torzewska, B. Bartodziejska, A.S. Shashkov, A. Rozalski, Y.A. Knirel, *Carbohydr. Res.*, 328 (2000) 229–234.
[9] Y.A. Knirel, E.V. Vinogradov, A.J. Mort, *Adv. Carbohydr. Chem. Biochem.*, 47 (1989) 167–202.
[10] N.P. Arbatsky, A.S. Shashkov, E. Literacka, G. Widmalm, W. Kaca, Y.A. Knirel, *Carbohydr. Res.*, 323 (2000) 81–86.
[11] O. Westphal, K. Jann, *Methods Carbohydr. Chem.*, 5 (1965) 83–91.