

Preliminary communication

2,3-Diacetamido-2,3-dideoxy-D-glucuronic acid: a new acidic amino sugar from *Pseudomonas aeruginosa* type O6 lipopolysaccharide

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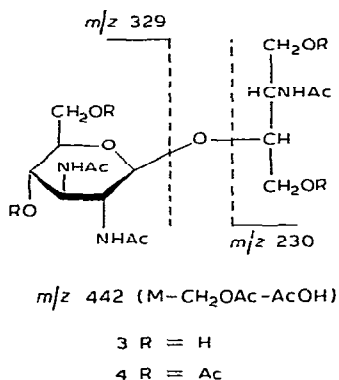
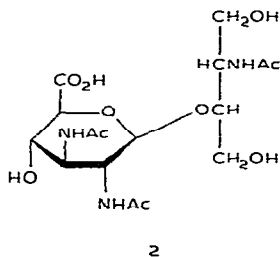
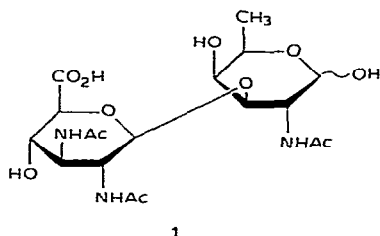
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The O-specific polysaccharide chains of *Ps. aeruginosa* lipopolysaccharides contain various amino sugars, some as yet unidentified¹. We now report the identification of 2,3-diacetamido-2,3-dideoxy-D-glucuronic acid as a constituent of the O-specific polysaccharide of *Ps. aeruginosa*, strain 170014 (serotype O6, Lanyi classification²). The acidic polysaccharide (M_{GalA} 0.65; paper electrophoresis; pyridine acetate buffer, pH 4.5), obtained by mild, acid degradation of the lipopolysaccharide isolated from dry bacterial cells by the Westphal procedure³, gave 2-amino-2-deoxygalactose, 2-amino-2-deoxyfucose, and 2-amino-2,6-dideoxyglucose on hydrolysis (2M HCl, 100°, 4 h), which were identified by conventional methods⁴. The ¹³C-n.m.r. spectrum of the polysaccharide contained signals for four anomeric carbons (103.3, 99.8, 99.2, and 97.9 p.p.m.), two C-methyl groups of 6-deoxyhexoses (17.4- and 16.6 p.p.m.), one hydroxymethyl group (60.8 p.p.m.), and five acetamido groups (lines for methyl and carbonyl at 23.3 and 174–176 p.p.m., respectively, in combination with those for carbons carrying nitrogen at 55.9, 55.7, 53.7, 50.9, and 49.1 p.p.m.). The spectrum of the carboxyl-reduced⁵ polysaccharide contained an additional signal for a hydroxymethyl group (61.5 p.p.m.). It is therefore proposed that the tetrasaccharide repeating-unit of the polysaccharide is composed of 2-acetamido-2-deoxygalactose, 2-acetamido-2-deoxyfucose, 2-acetamido-2,6-dideoxyglucose, and a diacetamidodideoxyuronic acid.

Treatment⁶ of the polysaccharide with hydrogen fluoride (25°, 3 h) gave the acidic disaccharide **1** (M_{GalA} 0.64 and R_{GalNac} 0.45; 1-butanol–pyridine–water, 6:4:3), which was isolated by gel filtration on Sephadex G-15 followed by preparative p.c. On hydrolysis, **1** gave 2-amino-2-deoxy-D-fucose hydrochloride, $[\alpha]_{\text{D}}^{20} +30^\circ$ (water), and the ¹³C-n.m.r. data were consistent with the structure assigned (**1**).

Treatment of **1**, in sequence, with borohydride, periodate, and borohydride gave the acidic glycoside (**2**), which was carboxyl-reduced⁵ to give **3**, the structure of which was indicated by ¹³C-n.m.r. data and the mass spectrum of the acetylated derivative **4**. On reduction of **2** to **3**, one ¹³C resonance was displaced from 71.1 to 68.9 p.p.m., which is characteristic⁷ of the behaviour of C-4 in the conversion of uronic acids into the corresponding hexoses. Thus, **2** and **3** contain a hydroxyl group at position 4, and,



consequently, the two acetamido groups are attached to positions 2 and 3. The coupling constants ($J_{1,2}$ 8.2, $J_{2,3}$ 11.5, $J_{3,4}$ 10.0, $J_{4,5}$ 10.0, $J_{5,6}$ 5.5, and $J_{5,6}^1$ 2.0 Hz) determined from the ^1H -n.m.r. spectrum of **4** indicated H-1,2,3,4,5 to be axial and the configuration of the diacetamidohexose residue to be β -gluco. Hydrolysis (4M HCl, 100°, 16 h) of **3** followed by chromatography on Dowex 50-X8 resin gave 2,3-diamino-2,3-dideoxy-D-glucose, the di-*N*-acetyl derivative of which had m.p. 252–254° (from methanol), $[\alpha]_{\text{D}}^{20}$ -49° (c 0.33, water); *cf.* m.p. 253–254°, $[\alpha]_{\text{D}}^{23}$ -46.1° (c 0.9, water), for the synthetic⁸ D-isomer.

Thus, the acidic amino component of the O-specific polysaccharide of *Ps. aeruginosa* type O6 is 2,3-diacetamido-2,3-dideoxy-D-glucuronic acid. Recently, we have found, in the O-specific polysaccharides of *Ps. aeruginosa* serogroup O3, two other representatives of this novel class of acidic amino sugars, which were tentatively identified as 2,3-diacetamido-2,3-dideoxy-D-mann- and -L-gul-uronic acids. These results will be described elsewhere.

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