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Preliminary communication

2,3-Diacetamido-2,3-dideoxy-D-mannuronic acid and its 2-imidazoline derivative: new acidic amino sugars from *Pseudomonas aeruginosa* O: 3a,d lipopolysaccharide

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2,3-Diacetamido-2,3-dideoxy-D-glucuronic acid has been identified[1] as a structural component of the *Ps. aeruginosa* O:6 O-specific polysaccharide. We now report the identification of 2,3-diacetamido-2,3-dideoxy-D-mannuronic acid and 2,3-(1-acetyl-2-methyl-2-imidazolino-5,4)-2,3-dideoxy-D-mannuronic acid as constituents of the O-specific polysaccharide of *Ps. aeruginosa*, strain 170005 (serotype O:3a,d, Lanyi classification).

The acidic polysaccharide (M_{GalA} 0.5; paper electrophoresis; pyridine acetate buffer, pH 4.5) was obtained by mild, acid degradation (1% CH_3CO_2H, 100°, 2 h) of the lipopolysaccharide isolated from dry bacterial cells by the Westphal procedure[2]. Hydrolysis (4M HCl, 100°, 4 h) of the polysaccharide followed by conventional sugar analysis resulted in identification of 2-amino-2-deoxy-D-fucose hydrochloride, $[\alpha]_D^{20}$ +60.5° (water), in 5% yield as a single monosaccharide. The ^{13}C-n.m.r. spectrum of the polysaccharide contained signals for three anomeric carbons (100.8, 99.8, and 98.3 p.p.m.), five carbons carrying nitrogen (57.6, 52.8, 52.4, 51.0, and 48.7 p.p.m.), one *C*-methyl group of a 6-deoxyhexose (16.4 p.p.m.), acetamido methyl groups at 23.1 p.p.m., and carbonyl groups in the region 174–176 p.p.m., as well as two signals at 19.7 and 167.1 p.p.m. subsequently assigned to *C*-methyl and C-2, respectively, of a 2-imidazoline derivative (see below). Signals for hydroxymethyl groups were absent from the spectrum. Therefore, it is proposed that the trisaccharide repeating-unit of the polysaccharide comprises 2-acetamido-2-deoxy-D-fucose and, probably, two diaminodideoxyuronic acid derivatives.

Solvolysis[3] of the polysaccharide with hydrogen fluoride (25°, 3 h) gave the acidic trisaccharide **1**, M_{GalA} 0.5, which was isolated by gel filtration on Sephadex G-15 in almost quantitative yield. The ^{13}C-n.m.r. spectrum of **1** showed that the solvolysis had cleaved selectively the *N*-acetylfucosaminidic linkages. Thus, **1** was the chemical repeating-unit of the polysaccharide. Treatment of **1**, in sequence, with borohydride, per-

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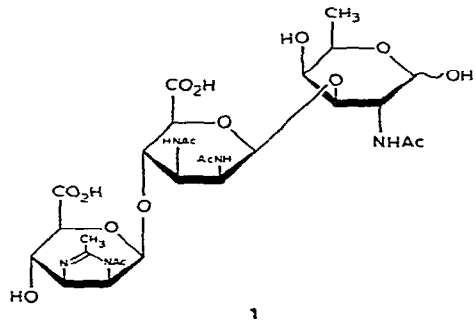

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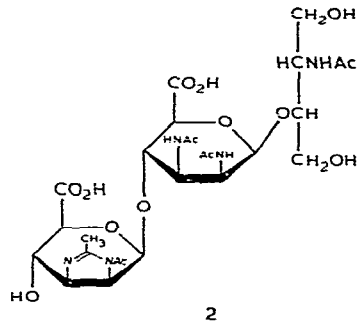

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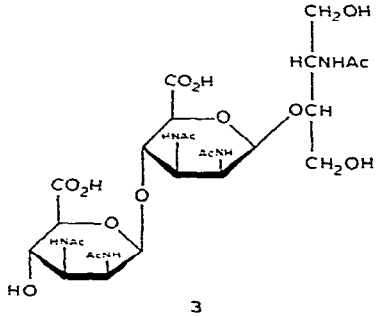

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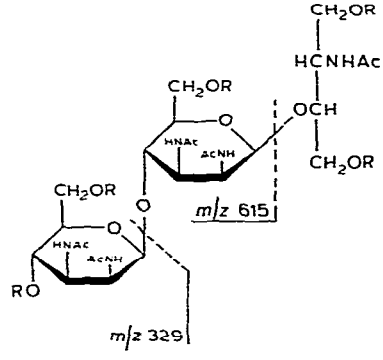

4 R = H

5 R = Ac

iodate, and borohydride destroyed the 2-acetamido-2-deoxy-D-fucose residue, to give an acidic oligosaccharide 2, which was converted into the acidic oligosaccharide 3 by treatment with 5% aqueous triethylamine (60°, 3 h). Carboxyl-reduction[4] of 3 gave the neutral oligosaccharide 4, which was characterised by ^{13}C-n.m.r. and 360-MHz ^{1}H-n.m.r. spectroscopy and by the mass spectrum of the acetylated derivative 5. In the ^{13}C-n.m.r. spectrum, the total number of signals and the number of signals for anomeric carbons and carbons carrying nitrogen indicated the presence of two diacetamidodideoxyhexose residues and the four-carbon aglycon in 4. The coupling constants ($J_{1,2}$ 1.5, $J_{2,3}$ 3.9, $J_{3,4}$ 9.8, $J_{4,5}$ 9.8, $J_{1',2'}$ 1.0, $J_{2',3'}$ 3.6, $J_{3',4'}$ 10.4, and $J_{4',5'}$ 10.0 Hz) determined from the ^{1}H-n.m.r. spectrum of 5 using homonuclear double-resonance proved H-3,4,5,3',4',5' to be axial and H-2,2' to be equatorial and, thus, the configuration of both hexose residues to be *manno*.

Hydrolysis (4M HCl, 100°, 3 h) of oligosaccharide 4 gave 2-amino-2-deoxythreitol and 2,3-diamino-2,3-dideoxymannose*, which were separated by ion-exchange

*Hydrolysis caused a partial epimerisation of 2,3-diamino-2,3-dideoxymannose into the *gluco* isomer.

chromatography on Dowex 50-X8 resin. *N*-Acetylation of the diamino sugar thus obtained gave 2,3-diacetamido-2,3-dideoxy-D-mannose, $[\alpha]_D^{20}$ –33.8° (water), which was practically identical by optical rotation, p.c. (R_F 0.48; 1-butanol–pyridine–water, 6:4:3), g.l.c.–mass spectrometry (OV-17), and ^{13}C-n.m.r. data with authentic material, $[\alpha]_D^{20}$ –38.0° (water), synthesised from methyl 2,3-diacetamido-4,6-*O*-benzylidene-2,3-dideoxy-α-D-mannopyranoside[5]. Comparison of the ^{13}C-n.m.r. spectra of oligosaccharide **4** and 2,3-diacetamido-2,3-dideoxy-α,β-D-mannopyranose showed that both hexose residues in **4** were β-linked.

Comparison of the ^{13}C-n.m.r. data for **4** and **3** revealed two hydroxymethyl signals (61.8 and 61.3 p.p.m.) in the spectrum of **4**; thus, **3** was composed of two 2,3-diacetamido-2,3-dideoxymannuronic acid residues. Further, in the ^{13}C-n.m.r. spectrum of **3**, as compared with the spectrum of **2**, substantial shifts of the signals at 167.1 and 19.7 p.p.m. into the regions for acetamido groups (175–176 and 23.1 p.p.m., respectively) were observed. The conversion of **2** into **3** also caused a marked increase in electrophoretic mobility (from M_{GalA} 0.55 to 0.9). Therefore **2** contains an *N*-acetylacetamidine function (having a basic nature) involving fused 2-imidazoline and pyranoid rings, with the former opening in basic conditions to give two acetamido groups. This inference was supported by the analogous behaviour of 1-acetyl-2-methyl-2-imidazoline[6], which reacted with water to give *N,N'*-diacetylethylenediamine and had a *C*-methyl resonance at 19.1 p.p.m. in its ^{13}C-n.m.r. spectrum.

Comparison of the ^{13}C-n.m.r. spectra of **3** and **4** with that of 2,3-diacetamido-2,3-dideoxy-β-D-mannopyranose allowed all of the signals of the terminal monosaccharide residue in the oligosaccharides to be assigned. In particular, the signal at 54.4 p.p.m. in the spectrum of **3** was unambiguously assigned to C-3 of the terminal diacetamidodideoxymannuronic acid residue. Comparison of the ^{13}C-n.m.r. spectra of **3** and **2** revealed a downfield shift (from 54.4 to 57.4 p.p.m.) of the foregoing C-3 signal, and the 2-imidazoline derivative thus occupies the terminal position in **2**. Furthermore, the substantial shift (3 p.p.m.) of the C-3 signal indicated the double bond of the 2-imidazoline ring to be located at the nitrogen at C-3 of the pyranoid ring, because the chemical shifts of the signals for other carbons carrying nitrogen differed, if at all, by not more than 1.5 p.p.m.

Thus, the O-specific polysaccharide of *Ps. aeruginosa* O:3a,d contains two acidic diamino sugars, namely, 2,3-diacetamido- and 2,3-(1-acetyl-2-methyl-2-imidazolino-5,4) 2,3-dideoxy-D-mannuronic acids, which have not been observed previously in Nature, and the structure of the chemical repeating-unit of the polysaccharide is the trisaccharide **1**.

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