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Preliminary communication

2,3-Diacetamido-2,3-dideoxy-L-guluronic acid: a new acidic amino sugar from *Pseudomonas aeruginosa* O:3a,d,e lipopolysaccharide

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The O-specific polysaccharides of *P. aeruginosa* O:3 (Lanyi classification) possess similar structures that are composed of trisaccharide repeating-units containing 2-acetamido-2-deoxy-D-fucose and uncommon, acidic, diamino sugars[1]. Recently, two of these sugars were identified as 2,3-diacetamido-2,3-dideoxy-D-mannuronic acid and its 2-imidazoline derivative[2]. We now report the identification of 2,3-diacetamido-2,3-dideoxy-L-guluronic acid as a constituent of *P. aeruginosa* O:3a,d,e (strain 170006) O-specific polysaccharide.

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[3]. Hydrolysis (4M HCl, 100°, 4 h) of the polysaccharide followed by conventional sugar analysis resulted in identification of 2-amino-2-deoxy-D-fucose in 3% yield as the single monosaccharide. The ^{13}C-n.m.r. spectrum of the polysaccharide contained signals for three anomeric carbons (100.0, 99.7, and 98.0 p.p.m.), five carbons carrying nitrogen (57.8, 51.3, 50.8, 49.7, and 45.0 p.p.m.), one *C*-methyl group of a 6-deoxyhexose (16.4 p.p.m.), four acetamido methyl groups in the region 23.0–23.7 p.p.m., and six carbonyl groups in the region 174–177 p.p.m., as well as signals at 19.8 and 167.2 p.p.m. belonging to *C*-methyl and C-2, respectively, of a 2-methyl-2-imidazoline derivative[2]. All other signals for the 2,3-(1-acetyl-2-methyl-2-imidazolino-5,4)-2,3-dideoxy-β-D-mannuronic acid residue (100.0, 76.3, 71.7, 57.8, and 51.3 p.p.m.) were also present in the spectrum. Signals for hydroxymethyl groups were absent. Therefore, it is proposed that the trisaccharide repeating-unit of the polysaccharide comprises 2-acetamido-2-deoxy-D-fucose, a diacetamidodideoxyuronic acid, and a 2-imidazoline derivative of a uronic acid probably having the *manno* configuration.

Solvolysis[4] 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, periodate, borohydride, and 5% aqueous triethylamine (60°, 3 h; to cleave the imidazoline ring)

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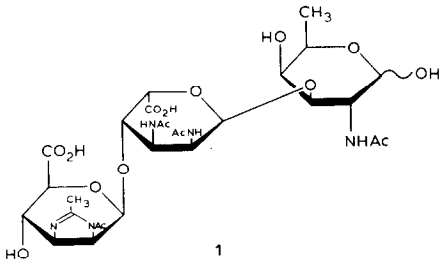

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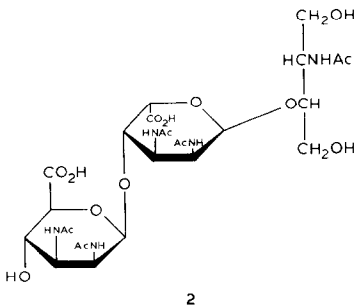

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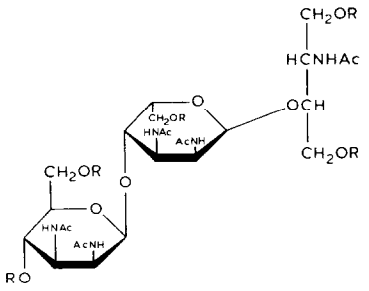

3 R = H

4 R = Ac

gave the acidic oligosaccharide **2**, M_{GalA} 0.9. Carboxyl-reduction[5] of **2** gave the neutral oligosaccharide **3**, which was further acetylated to give **4**.

The coupling constants ($J_{1',2'}$ 1.5, $J_{2',3'}$ 3.6, $J_{3',4'}$ 10.0, and $J_{4',5'}$ 10.0 Hz) determined from the 360-MHz ^{1}H-n.m.r. spectrum of **4** proved H-3′,4′,5′ to be axial and H-2′ to be equatorial and, thus, the configuration of the terminal hexose residue to be *manno*. The only coupling constants that could be determined for the second hexose residue were $J_{1,2}$ 3.5 and $J_{4,5}$ 2.6 Hz, the latter indicating H-4 to be equatorial. Comparison of the ^{13}C-n.m.r. spectra of **3** and the analogous oligosaccharide obtained from the *P. aeruginosa* O:3a,d O-specific polysaccharide[2] revealed the presence of the same aglycon (2-acetamido-2-deoxythreitol) and terminal residue (2,3-diacetamido-2,3-dideoxy-β-mannopyranose) and allowed their signals to be assigned. The remaining six signals in the spectrum of **3** (99.4, 76.0, 68.2, 61.8, 50.1, and 45.6 p.p.m.) should be assigned, therefore, to the penultimate hexose residue, those at 99.4 and 61.8 p.p.m. belonging unambiguously to C-1 and C-6, respectively. Furthermore, the signal at 76.0 p.p.m. was assigned to C-4, according to its downfield shift in the spectrum of **2** (to 77.4 p.p.m.), which is characteristic for the conversion of a hexose into the corresponding uronic acid[6]. Thus, the carbons carrying acetamido groups were C-2 and C-3 (signals at 45.6 and 50.1 p.p.m.). The remaining signal at 68.2 p.p.m. was consequently assigned to C-5. The C-1–H-1 coupling constant (J 172.0 Hz, determined from the ^{13}C-n.m.r. spectrum of the polysaccharide) indicated the unknown 2,3-diacetamido-2,3-dideoxyuronic acid to be α-linked, assuming its $^4C_1$ conformation[7,8]. Therefore, the ^{13}C-n.m.r. data for **3** were compared with those for all eight methyl 2,3-diacetamido-2,3-dideoxy-α-D-hexopyrano-

sides*. The comparison revealed the similarity in the chemical shifts of the signals for C-2 and C-5 in the spectra of **3** (45.6 and 68.2 p.p.m.) and methyl 2,3-diacetamido-2,3-dideoxy-α-D-gulopyranoside (45.4 and 68.0 p.p.m., respectively), and differences from the values for the other hexosides (⩾3.5 p.p.m. for C-2 and 1 p.p.m. for C-5). Thus, the configuration of the unknown sugar is *gulo*.

Attempts to isolate 2,3-diamino-2,3-dideoxygulose by acid hydrolysis of **3** were unsuccessful due to its instability, which was supported by model experiments with methyl 2,3-diacetamido-2,3-dideoxy-α-D-gulopyranoside. In order to determine the absolute configuration of the new sugar, the dependence of the glycosidation shifts of aglycon carbon signals in the ^{13}C-n.m.r. spectra on the absolute and anomeric configuration of the glycon[9] was used. The relatively low (by module) effect (−1.4 p.p.m.) on C-4 of the 2-acetamido-2-deoxy-D-fucosyl residue in the spectrum of the polysaccharide caused by substitution at position 3 by a 2,3-diacetamido-2,3-dideoxy-α-gulopyranosyluronic acid residue indicated the L configuration of the sugar substituent. This assignment was supported by the $[\alpha]_D$ value of −66.7° (water) for **3**.

Thus, the O-specific polysaccharide of *P. aeruginosa* O:3a,d,e contains 2,3-diacetamido-2,3-dideoxy-L-guluronic acid, which has not been observed previously in Nature, and the structure of the chemical repeating-unit of the polysaccharide is the trisaccharide **1**.

REFERENCES

1 Yu. A. Knirel, E. V. Vinogradov, and B. A. Dmitriev, *Abstr. Pap. Gen. Appl. Chem. Mendeleev's Meeting, XIIth*, Moscow, 1981, pp. 131–133.
2 Yu. A. Knirel, E. V. Vinogradov, A. S. Shashkov, B. A. Dmitriev, and N. K. Kochetkov, *Carbohydr. Res.*, 104 (1982) C4–C7.
3 O. Westphal and K. Jann, *Methods Carbohydr. Chem.*, 5 (1965) 83–91.
4 A. J. Mort and D. A. Lamport, *Anal. Biochem.*, 82 (1977) 289–309.
5 R. L. Taylor, J. E. Shively, and H. E. Conrad, *Methods Carbohydr. Chem.*, 7 (1976) 141–151.
6 P. A. J. Gorin and M. Mazurek, *Can. J. Chem.*, 53 (1975) 1212–1223.
7 K. Bock and C. Pedersen, *J. Chem. Soc., Perkin Trans. 2*, (1974) 293–297.
8 S. J. Angyal, *Aust. J. Chem.*, 21 (1968) 2734–2746.
9 A. S. Shashkov, A. I. Usov, Yu. A. Knirel, B. A. Dmitriev, and N. K. Kochetkov, *Bioorg. Khim.*, 7 (1981) 1364–1371.

*The synthesis of these compounds will be described elsewhere.