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Preliminary communication

The structure of the *Pseudomonas aeruginosa* immunotype 6 O-antigen: isolation and identification of 5-acetamido-3,5,7,9-tetradeoxy-7-formamido-L-*glycero*-L-*manno*-nonulosonic acid

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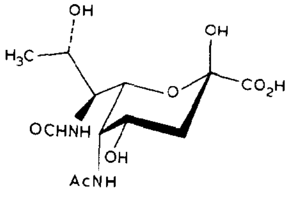
Derivatives of 5,7-diamino-3,5,7,9-tetradeoxynonulosonic (pseudaminic) acid have been found[1] in some bacterial lipopolysaccharides (LPS), and one of them has been identified as 5-acetamido-3,5,7,9-tetradeoxy-7-[(*R*)-3-hydroxybutyramido]-L-*glycero*-L-*manno*-nonulosonic acid[2]. We now report the identification of 5-*N*-acetyl-7-*N*-formylpseudaminic acid (**1**) which is the first representative of these new sialic acid-like sugars isolated in a free state. The structure of the O-specific side-chain of *P. aeruginosa* immunotype 6 LPS containing **1** has also been established.



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The LPS was isolated from dry bacterial cells by phenol–water extraction[3]. The ^{13}C-n.m.r. data indicated it to have the same monosaccharide composition as the LPS from *P. aeruginosa* O5a,b,d, including *N*-acetyl-*N'*-formylpseudaminic acid, xylose, and 2-acetamido-2,6-dideoxygalactose[1]. The last two sugars, which were found earlier[4] to be the components of this LPS, were isolated after mild acid hydrolysis of the LPS (1% HOAc, 100°, 1.5 h) followed by solvolysis with HF (20°, 3 h) and were identified as D isomers on the basis of their optical rotations. Both aldoses are present in the pyranoid form and are β (105.5 and 99.4 p.p.m., both C-1, $^1J_{C,H}$ 162 and 163 Hz*, respectively)[5].

On solvolysis with HF in methanol[2,6] (20°, 2 h), the LPS gave the methyl glycosides of disaccharides **2** and **3**, as well as of trisaccharides **4**–**6**: α-PseN$_2$AcFm-(2→4)-β-D-Xyl*p*-(1→OMe (**2**), α-PseN$_2$AcFm-(2→4)-α-D-Xyl*p*-(1→OMe (**3**), α-PseN$_2$AcFm-(2→4)-β-D-Xyl*p*-(1→3)-β-D-FucNAc*p*-(1→OMe (**4**), α-PseN$_2$AcFm-(2→4)-β-D-Xyl*p*-(1→3)-α-D-FucNAc*p*-(1→OMe (**5**), and α-PseN$_2$AcFm-(2→4)-β-D-Xyl*p*-(1→3)-β-D-FucNAc*f*-(1→OMe

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*Determined from the gated-decoupling spectrum.

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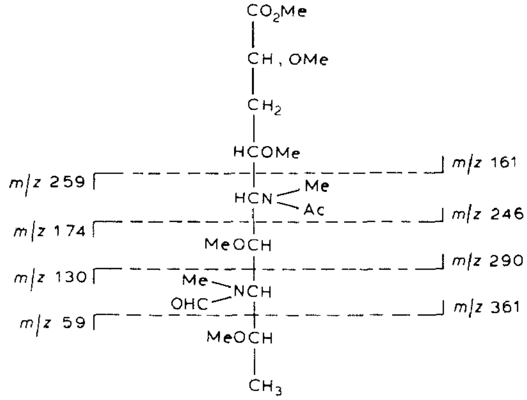
(**6**), where PseN$_2$AcFm is **1**. The oligosaccharides were identified by using ^{13}C-n.m.r. spectroscopy, the spectra being interpreted by comparison with each other and with those of the LPS and **1** (see below).

Analysis of the glycosidation effects in the spectra gave information on the positions of the glycosidic linkages[7]. Thus, in the spectrum of **2**, the signals for C-3,5 of the xyloside residue were displaced by −1.6 and −2.0 p.p.m., respectively, as compared with those for methyl β-D-xylopyranoside[8], which is characteristic of substitution of this sugar in position 4. The small α-effect (+1.4 p.p.m.) for the signal of C-4 of xylose also accords with ketosidic substitution at O-4. 2-Acetamido-2,6-dideoxygalactose is glycosidated at O-3, as follows from the low-field position of the signal for C-3 at 81.3 p.p.m. in the spectrum of the LPS. Finally, comparison of the chemical shifts for the signals of C-3,4,5 of pseudaminic acid in **2** (36.8, 66.3, and 49.9 p.p.m.) and the LPS (35.4, 72.4, and 46.5 p.p.m.) showed this sugar to be substituted at position 4 in the LPS and to occupy the non-reducing end in the oligosaccharide. These data also revealed the sequence of the monosaccharides in the repeating unit.

Formation of the oligosaccharides **2**–**6** proved the glycosidic linkage of pseudaminic acid to be more stable towards HF than those of xylose and 2-acetamido-2,6-dideoxygalactose. In contrast, this linkage was extremely labile towards acid, and mild hydrolysis (1% HOAc, 100°, 2 h) of **2**–**6** yielded **1** which was isolated by ion-exchange chromatography on DEAE-Trisacryl M followed by preparative p.c. (ethyl acetate–pyridine–acetic acid–water 5:5:1:3, R_{NeuNAc} 1.0). Compound **1** had $[\alpha]_D^{20}$ −38° (water). N.m.r. data: ^{1}H, δ 1.08 (d, 3 H, $J_{8,9}$ 6.6 Hz, H-9), 1.77 (dd, 1 H, $J_{3a,3e}$ 13.5, $J_{3a,4}$ 12.3 Hz, H-3*a*), 1.89 (dd, 1 H, $J_{3e,4}$ 5.5 Hz, H-3*e*); ^{13}C, 177.3, 97.6, 35.8, 66.7, 49.9, 71.1, 52.7, 67.6, and 16.3 p.p.m. for C-1,2,3,4,5,6,7,8,9, respectively. Reduction of **1** with NaBH$_4$ and methylation[9] of the product gave **7**. Analysis of the mass spectrum of **7** revealed the positions of the amino groups, the deoxy groups, and the *N*-acyl substituents (acetyl at N-5 and formyl at N-7).



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The H,H coupling constants $J_{3a,4}$ 12.3, $J_{4,5}$ 4, and $J_{5,6}$ 2 Hz for **1** indicated an axial,equatorial orientation of H-4,5 and thus proved the *lyxo* configuration of the fragment C-4,5,6. The similar large $J_{6,7}$ values (10.6 and 10.0 Hz, respectively) for **1** and the derivative of pseudaminic acid from *P. aeruginosa* O10a O-specific polysaccharide[1] proved the C-6,7 fragment in each sugar to have the *erythro* configuration[2] (a $J_{6,7}$ value of 1.2 Hz has been reported[10] for *N*-acetylneuraminic acid in which this fragment has the *threo* configuration). The *erythro* configuration of the C-7,8 fragment followed from the relatively high-field position (16.3 p.p.m.) of the signal for C-9 of **1**, whereas C-9 of the *threo* isomer should resonate near 20 p.p.m. (*cf.* the data[11] for C-4 of *N*-acetylallothreonine and *N*-acetylthreonine). Thus, **1** has the same general configuration as the L-*glycero*-L-*manno* isomer in the *P. aeruginosa* O10a O-specific polysaccharide[2], which is confirmed also by the similar ^{13}C chemical shifts for the signals of C-3,4,5,7,8,9 of both sugars (*cf.* the data in ref. 1).

The relative absolute configuration of **1** was established by ^{13}C-n.m.r. spectroscopy using the known regularities for glycosidation effects[12]. The situation in the disaccharide unit β-D-FucNAc*p*-(1→4)-PseN$_2$AcFm is favorable because of the relatively small α-effects for C-1 of 2-acetamido-2,6-dideoxy-β-D-galactose and for C-4 of pseudaminic acid (+3.3 and +6.0 p.p.m., respectively), as well as the relatively large (by module) β-effect (−3.4 p.p.m.) for C-5 of the latter sugar (the effects were determined by comparison of the ^{13}C-n.m.r. data for the LPS and the corresponding free sugars). These data are characteristic of a disaccharide unit β-D-Hex*p*-(1→3)-L-Gal[12], the lack of a hydroxyl group at C-3 of pseudaminic acid (corresponding to C-2 of galactose) thus being of little significance. Therefore, the fragment C-4,5,6 of **1** is homomorphic with the fragment C-3,4,5 of L-galactose (but not of D-galactose); hence the new sugar has the L-*glycero*-L-*manno* configuration.

The relatively small difference (~0.6 p.p.m.) between the chemical shifts of the signals for H-3*e* and H-3*a* of pseudaminic acid in **2**–**6** is characteristic of the equatorial orientation of the carboxyl group[13] (*cf.* the difference of 0.9 p.p.m. for the pseudaminic acid having an axial carboxyl group in the *P. aeruginosa* O10a O-specific polysaccharide[1]). Therefore, this sugar is α in **2**–**6** in accordance with the rules of carbohydrate nomenclature[14]. The higher-field position (~72 p.p.m.) of the signal for C-6 of pseudaminic acid in **2**–**6**, as compared with that (75 p.p.m.) in the *P. aeruginosa* O10a polysaccharide[1], confirmed this conclusion[15]. There is only one series of signals for one of the anomers in the ^{1}H- and ^{13}C-n.m.r. spectra of **1**, and since the chemical shifts for the signals of C-6 in **1** and in **2**–**6** are similar, cleavage of the glycosidic linkage of pseudaminic acid was not accompanied by anomerisation.

Thus, the repeating unit of the polysaccharide chain of *P. aeruginosa* immunotype 6 LPS is →4)-α-PseN$_2$AcFm-(2→4)-β-D-Xyl*p*-(1→3)-β-D-FucNAc*p*-(1→.

According to the ^{13}C-n.m.r. data, the O-specific polysaccharide of *P. aeruginosa* O5a,d (Lányi classification) also has this structure, whereas those of the serotypes O5a,b,d and O5a,b,c differ by the presence of an *O*-acetyl group at position 4 of the 2-acetamido-2,6-dideoxygalactose residue. Also, the polysaccharide of serotype O5a,b,c has an (*R*)-3-hydroxybutyryl group at N-5 of pseudaminic acid instead of an acetyl group[1].

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