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Preliminary communication

Identification of 5-acetamido-3,5,7,9-tetradeoxy-7-[(*R*)-3-hydroxybutyramido]-L-*glycero*-L-*manno*-nonulosonic acid as a component of bacterial polysaccharides

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Recently, in some O-specific polysaccharides of *Shigella boydii* and *Pseudomonas aeruginosa*, we found new sialic acid-like sugars, which were derivatives of 5,7-diamino-3,5,7,9-tetradeoxynonulosonic (pseudaminic) acid[1]. We now report the determination of the configuration of pseudaminic acid, as well as of its acyl substituent at N-7, namely, the 3-hydroxybutyryl group.

Two and three successive Smith-degradations of the *S. boydii* type 7 polysaccharide, containing[1] D-glucose, D-galactose, 2-acetamido-2-deoxy-D-glucose, and di-*N*-acyl-pseudaminic acid in the ratios 1:2:1:1, afforded oligosaccharides **1** and **2**, respectively. Oligosaccharide **2** was converted into the acetylated methyl ester **3**, the ^{1}H-n.m.r. spectrum of which was fully interpreted by using homonuclear double resonance. The coupling constants $J_{3a,4}$ 13, $J_{4,5}$ 3.5, and $J_{5,6}$ 1.6 Hz indicated the axial orientation of H-4 and the equatorial orientation of H-5 of pseudaminic acid. The equatorial orientation of the substituent at C-6 was supported by the considerable n.O.e. (2.5%) for H-7 on pre-irradiation of the proton at N-5. The n.O.e. for H-3*a* (2%) confirmed the axial orientation of the substituent at C-5. Thus, the fragment C-4,5,6 has the *lyxo* configuration.

The large value (~10 Hz) of $J_{6,7}$ indicated the preponderant occurrence of rotamer **4** having the antiperiplanar orientation of H-6 and H-7. The considerable n.O.e. for H-5 and H-6 (~3%) observed on pre-irradiation of the proton at N-7 proved the *erythro* configuration of the fragment C-6,7 (*cf.* $J_{6,7}$ 1.2 Hz for neuraminic acid[2], having the *threo* configuration of C-6,7).

Comparison of the ^{13}C-n.m.r. data for glycoside **2** and oligosaccharide **1** showed the 2-acetamido-2-deoxy-D-glucose in **1** to be α-linked (δ_C 95.7, C-1, $^1J_{CH}$ 171 Hz†,

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†Determined from the gated-decoupling spectrum.

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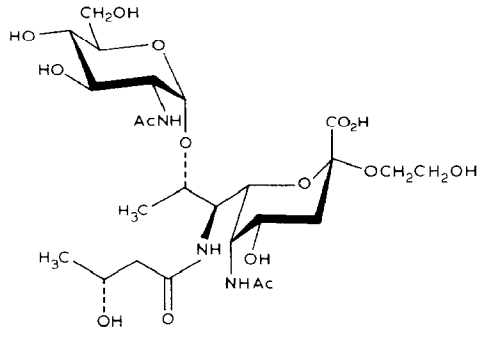

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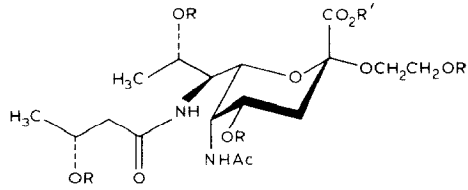
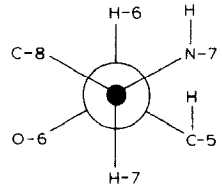


2 R = R′ = H
3 R = Ac, R′ = Me



4

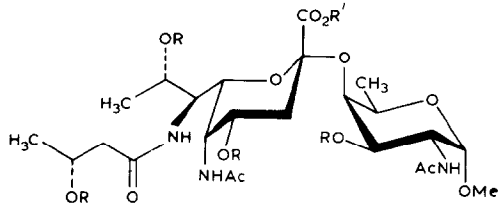
ref. 3) to O-8 of pseudaminic acid (the glycosidation shifted the signals of C-7,8,9 from 54.6, 69.9, and 18.0 p.p.m. in **2** to 54.0, 73.3, and 14.0 p.p.m., respectively, in **1**). Comparing the values of the β-effect for C-9 of pseudaminic acid (−4 p.p.m.) and the α-effect for C-1 of 2-acetamido-2-deoxy-D-glucose (+3.4 p.p.m., as determined using literature data[4]) with the corresponding ^{13}C-n.m.r. data for model compounds (D- and L-threonine, D- and L-allothreonine, and their *O*-glycosylated derivatives[5]), the fragment C-7,8,9 of pseudaminic acid was concluded to be homomorphic to the C-2,3,4 fragment of L-allothreonine. Therefore, the C-7,8 fragment has the L-*erythro* configuration, and thus pseudaminic acid has the L-*glycero*-L-*manno* configuration.

The position of the H-3*e* signal of pseudaminic acid (2.46 p.p.m.) in the ^{1}H-n.m.r. spectrum of **2** indicated the carboxyl group to be axial[6], and thus the glycosidic linkage of this sugar has the β configuration, according to the rules[7] of carbohydrate nomenclature.

Solvolysis of *P. aeruginosa* 010a** polysaccharide, containing[1] 2-acetamido-2,6-dideoxy-D-glucose, 2-acetamido-2,6-dideoxy-D-galactose, and di-*N*-acylpseudaminic acid in the ratios 1:1:1, with HF in methanol yielded disaccharide **5**. The latter contained, in addition to the pseudaminic acid derivative, methyl 2-acetamido-2,6-dideoxy-α-D-galactopyranoside substituted at position 4 [δ_C 99.3 (C-1) and 76.3 p.p.m. (C-4, *cf.* the data for methyl 2-acetamido-2,6-dideoxy-α-L-galactopyranoside[8]]. As described above for **3**, a study of the acetylated methyl ester **6** of **5** indicated that the sialic acid-like sugar in **5** has the same general and anomeric configuration as in **2**. This conclusion was also supported by the close agreement of the chemical shifts of their signals in the ^{13}C-n.m.r. spectra[1]. The absolute configuration of pseudaminic acid was also the same in

**Lányi classification.

both oligosaccharides, because of its negative contribution in the optical rotations of **2**, $[\alpha]_D$ −30° (water), and **5**, $[\alpha]_D$ ~0° (water) {methyl 2-acetamido-2,6-dideoxy-α-D-galactopyranoside has $[\alpha]_D$ +179° (water)[9], and, hence, is a positively contributing unti of **5**}.



5 R = R' = H
6 R = Ac, R' = Me

Hydrolysis of *S. boydii* and *P. aeruginosa* polysaccharides, followed by extraction of the hydrolysate with ethyl acetate, afforded (*R*)-3-hydroxybutyric acid, identified by the ^{1}H- and ^{13}C-n.m.r. data [δ_H 4.25 (sextet, 1 H, $J_{2,3} = J_{3,4}$ = 6.5 Hz, H-3), 2.50 (d, 2 H, H-2), 1.27 (d, 3 H, H-4); δ_C 180.0 (C-1), 66.2 (C-3), 44.8 (C-2), 23.5 (C-4)], as well as by the optical rotations, $[\alpha]_D$ −18° (*c* 0.3, water), for each product {lit.[10] $[\alpha]_D$ −24.5° (water)}.

Thus, the O-specific polysaccharides of *S. boydii* type 7 and *P. aeruginosa* O10a involve 5-acetamido-3,5,7,9-tetradeoxy-7-[(*R*)-3-hydroxybutyramido]-L-*glycero*-L-*manno*-nonulosonic acid. Its presence accounts for the strong serological cross-reaction between these strains[11], despite the lack of any common oligosaccharide fragments in the O-specific polysaccharides. The di-*N*-acyl derivatives of pseudaminic acid have also been found in the *P. aeruginosa* O5 polysaccharides[1], whereas in the *P. aeruginosa* O13 polysaccharide we have identified 5,7-diacetamido-3,5,7,9-tetradeoxy-D-*glycero*-L-*galacto*-nonulosonic acid (these data will be published elsewhere).

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