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Preliminary communication

Sialic acids of a new type from the lipopolysaccharides of *Pseudomonas aeruginosa* and *Shigella boydii*

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All sialic acids hitherto found in Nature are derivatives of 5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulosonic (neuraminic) acid[1]. We now report the identification of sialic acids of a new type, in lipopolysaccharides from *P. aeruginosa* O5 and O10 (Lányi classification[2]) and *Sh. boydii* type 7, as derivatives of 5,7-diamino-3,5,7,9-tetradeoxynonulosonic acid (pseudaminic acid). The new sialic acids were not isolated in a free state and their structures were established by ^{1}H- and ^{13}C-n.m.r. spectroscopy of oligosaccharides derived from the lipopolysaccharides.

The lipopolysaccharides, isolated from dry bacterial cells of *P. aeruginosa* O5a,b,c and O5a,b,d by the Westphal procedure[3], reacted with a resorcinol reagent to give a chromophore identical to that formed from *N*-acetylneuraminic acid[4]. In accordance with the earlier observation[5], mild acid hydrolysis (1% CH_3CO_2H, 100°, 1.5 h) of the lipopolysaccharides degradated the O-specific polysaccharide chains, and oligosaccharides **1** and **2** were subsequently isolated by gel filtration on Sephadex G-50 and were acidic in electrophoresis. On solvolysis with HF (20°, 3 h), the oligosaccharides yielded D-xylose and 2-acetamido-2,6-dideoxy-D-galactose[‡], which were identified by conventional methods, an acidic component being undetected.

The ^{1}H- and ^{13}C-n.m.r. data showed that **1** and **2** were trisaccharides containing xylose, 2-acetamido-2,6-dideoxygalactose, and a 3-deoxyaldulosonic acid [δ_C*[†] 99.7 (s, C-2) and 34.8 (t, C-3); δ_H 2.07 (dd, $J_{3e,4}$ 5, $J_{3e,3a}$ 12 Hz, H-3*e*) and 1.91 (t, $J_{3a,4}$ 12 Hz, H-3*a*)]. Unambiguous assignment of all signals in the ^{1}H-n.m.r. spectra of **1** and **2** was performed by homonuclear double-resonance, and all signals in their ^{13}C-n.m.r. spectra were then assigned by selective heteronuclear ^{13}C{^{1}H} double-resonance. The data obtained indicated that the xylose and 2-acetamido-2,6-dideoxygalactose residues in **2** were β-linked [δ_C* 105.4 and 99.6 ($^1J_{CH}$ 162 and 163 Hz, respectively[7], C-1), δ_H 4.37 and 4.72 (2 d, each $J_{1,2}$ 8 Hz, H-1)], and thus the aldulosonic acid was the reducing residue. The chemical shifts for xylose in the ^{13}C-n.m.r. spectrum of **2** were very similar to those reported for methyl β-D-xylopyranoside[8]; therefore, the xylosyl group occupied the terminal position.

[‡]Previously, these sugars were found to be the components of serologically related *P. aeruginosa* types 7 and 8 (Habs classification)[5] as well as immunotype 6 (Fischer)[6] lipopolysaccharides.
[†]The δ_C* data refer to gated-decoupling spectra.

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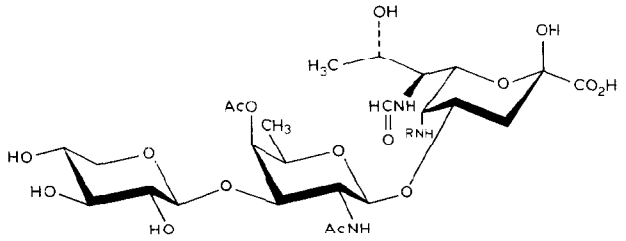
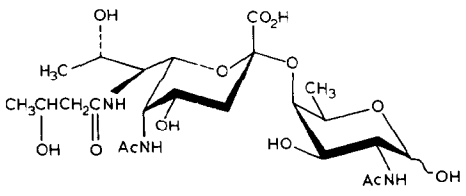


1 R = CH_3CHCH_2CO (OH)

2 R = Ac



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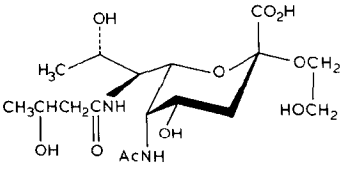

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Further, the 2-acetamido-2,6-dideoxygalactosyl residue was substituted by xylose at position 3 [δ_C 78.9 (C-3)] and by an O-acetyl group at position 4 [δ_H 5.24 (H-4)].

The ^1H and ^{13}C chemical shifts for the aldulosonic acid in **2** indicated it to be a diaminotetradeoxynonulosonic acid, the amino groups being attached to positions 5 and 7 (δ_C 46.6 and 52.3) and two deoxy groups occupying positions 3 (see above) and 9 [δ_C 16.3; δ_H 1.10 (d, 3 H, $J_{8,9}$ 6 Hz, H-9)]. One of the amino groups of the sialic acid residue in **2** was acetylated (δ_C 23.5, δ_H 1.98, CH_3CON), whereas the other carried a formyl group [δ_C* 164.9 (d, J_{CH} 197 Hz); δ_H 8.05 (s)]. The new sugar was substituted by 2-acetamido-2,6-dideoxygalactose at O-4 (δ_C 72.3, C-4), whereas HO-8 was unsubstituted (δ_C 67.5, C-8).

Comparison of the ^{13}C-n.m.r. spectra of **1** and **2** indicated that they differed only by the presence of an *N*-(3-hydroxybutyryl) group on the sialic acid residue in **1** [δ_C 65.9 (CHOH), 45.5 (t, CH_2), 23.5 (CH_3); δ_H 2.21 (d, J 6 Hz, CH_2) and 1.39 (d, J 6 Hz, CH_3)] instead of an *N*-acetyl group in **2**. The change of the *N*-acyl substituent caused distinct shifts of the signals for C-1 of the 2-acetamido-2,6-dideoxygalactosyl and C-4 of the sialic acid residues, from 99.6 and 72.3 p.p.m. in **2** to 100.6 and 73.2 p.p.m., respectively, in **1**, whereas the position of all other signals remained unaltered. The above shifts were indicative of the location of the substituent near the glycosidic linkage between these residues, *i.e.*, at C-5 of sialic acid, and thus the formamido group was attached to C-7.

Analysis of the ^{13}C-n.m.r. spectra of the corresponding lipopolysaccharides showed that trisaccharides **1** and **2** represent their chemical repeating-units and that the sialic acid residues in **1** and **2** existed almost completely in the same anomeric form as in the lipopolysaccharides.

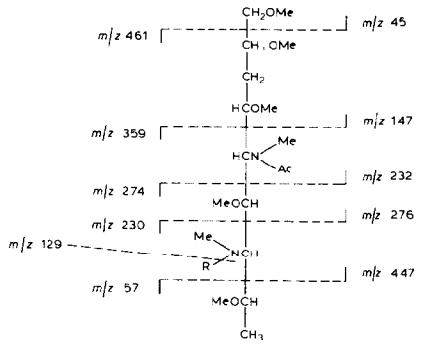
Lipopolysaccharides from *P. aeruginosa* O10a and *Sh. boydii* type 7 also contained an unusual sialic acid, but, in contrast to the above lipopolysaccharides, they gave the corresponding polysaccharides on hydrolysis, which were isolated by gel filtration on

Sephadex G-50. The results of acid hydrolysis, solvolysis with HF, and ^{1}H- and ^{13}C-n.m.r. spectroscopy proved in *P. aeruginosa* O10a polysaccharide to be composed of trisaccharide repeating-units made up of 2-acetamido-2,6-dideoxy-D-glucose, 2-acetamido-2,6-dideoxy-D-galactose, and an *N*-acetyl-*N'*-(3-hydroxybutyryl)diaminotetradeoxynonulosonic acid, while the *Sh. boydii* type 7 polysaccharide possesses a pentasaccharide repeating-unit containing D-glucose, D-galactose, 2-acetamido-2-deoxy-D-glucose, and the same sialic acid, in the ratios 1:2:1:1.

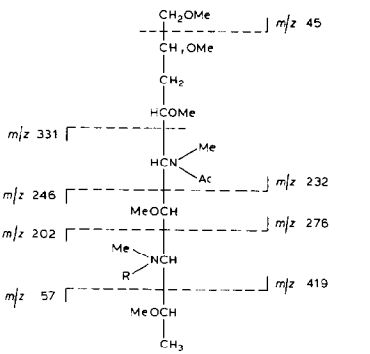
Solvolysis of *P. aeruginosa* O10a polysaccharide with HF (20°, 3 h) yielded N-acetyl-6-deoxyhexosamines and an oligosaccharide **3** (isolated by gel filtration on Sephadex G-15). Assignment of the signals in the ^{1}H- and ^{13}C-n.m.r. spectra (as above) showed that **3** was a disaccharide, the 2-acetamido-2,6-dideoxygalactose residue being at the reducing end (the spectra contained two series of signals corresponding to the α and β forms) and the sialic acid occupying the terminal position [δ_C* 102.6 (s, C-2)]. The ^{13}C chemical shifts for the sialic acid in **3** (δ_C 37.1, 67.7, 49.1, 75.0, 54.7, 69.3, and 17.7 for C-3,4,5,6,7,8,9) showed the same distribution of functional groups as for the sialic acid residues in **1** and **2**, while the similarity of the ^{1}H coupling constants ($J_{3e,4}$ 5, $J_{3a,4}$ 12, $J_{4,5}$ <1, $J_{5,6}$ 1.5, $J_{6,7}$ 10, $J_{7,8}$ 6, and $J_{8,9}$ 6 Hz) proved the configuration of the sialic acid residues in **1**, **2**, and **3** to be the same.

Some differences in chemical shifts of the signals for C-3,4,5,7,8 of the sialic acid residues in **2** and **3** were associated with glycosidation at C-4 in **2** and replacement of the formyl group at N-7 in **2** by an acetyl or 3-hydroxybutyryl group in **3**. The relatively high-field position of the signal for C-6 of the sialic acid in **2** (71.2 p.p.m.), as compared to that (75.0 p.p.m.) in **3**, is characteristic of a different anomeric configuration of these sugars (axial orientation of the carboxyl group in **3** and equatorial in **2**[9]). This conclusion was also supported by the chemical shifts for H-3*e* in the ^{1}H-n.m.r. spectra of **3** (δ 2.55) and **2** (δ 2.07)[10].

Sh. boydii type 7 polysaccharide was subjected to three successive Smith-degradations to give glycoside **4** which, according to the ^{13}C-n.m.r. data, was composed of sialic acid [δ_C* 101.8 (s, C-2)] and ethylene glycol as the aglycon (δ_C 66.8 and 61.8). Similar chemical shifts for C-3,4,5,6,7,8,9 in the ^{13}C-n.m.r. spectra of **3** and **4**, as well as the similarity of the ^{1}H coupling constants for the acetylated derivatives of **3** and **4**, showed that these oligosaccharides contained the same sialic acid. This finding is in agreement with the strong serological cross-reaction[11] between *P. aeruginosa* O10a and *Sh. boydii* type 7.



5 R = $COCH_2CH(OAc)CH_3$



6 R = $COCH_2CH(OMe)CH_3$

The position of the *N*-acyl substituents, as well as the whole structure of the sialic acid residues, was evident from the mass spectra of the derivatives **5** and **6**, which were prepared as follows. *P. aeruginosa* O10a and *Sh. boydii* type 7 polysaccharides were carboxyl-reduced[12] and then hydrolysed (0.01 M HCL, 100°, 2 h) to split the glycosidic linkage of the sialic acid residue, and the products were reduced with sodium borohydride. The oligosaccharide thus obtained from the former polysaccharide was methylated[13] and then solvolysed with HF, and the product was acetylated to give **5**. The oligosaccharide derived from the latter polysaccharide was solvolysed with HF and the product was methylated[13] to form **6**. The *O*-acetylation of the *N*-(3-hydroxybutyryl) group in **5** proved it to be glycosylated in the corresponding polysaccharide, whereas the sialic acid residue was unsubstituted.

Thus, the new sialic acids are derivatives of the same 5,7-diamino-3,5,7,9-tetradeoxynonulosonic (pseudaminic) acid. Determination of the configuration of pseudaminic acid (tentatively identified as L-*glycero*-L-*manno*) and of the 3-hydroxybutyryl residue is in progress.

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