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Preliminary communication

Structural studies of the *Pseudomonas aeruginosa* immunotype 1 antigen, containing the new sugar constituents 2-acetamido-2-deoxy-D-galacturonamide and 2-deoxy-2-formamido-D-galacturonic acid

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Two groups of new amino sugars, namely, 2,3-diacetamido-2,3-dideoxyuronic acids[1–3] and 5,7-diacylamino-3,5,7,9-tetradeoxynonulosonic acids[4], have been found as components of different *P. aeruginosa* lipopolysaccharides. We now report the identification of 2-acetamido-2-deoxy-D-galacturonamide and 2-deoxy-2-formamido-D-galacturonic acid as constituents of the *P. aeruginosa* immunotype 1 lipopolysaccharide.

The lipopolysaccharide was isolated from dry bacterial cells by the Westphal procedure[5] and degraded with 1% CH_3CO_2H (100°, 2 h) to give the O-specific polysaccharide (PS1). The ^{13}C-n.m.r. spectrum of PS1 was almost identical to that reported[6]; it was complicated by the presence of signals of low intensity caused by non-stoichiometric amounts of *O*-acetyl groups and was difficult to interpret. The ^{13}C-n.m.r. spectrum of the *O*-deacetylated (5% Et_3N, 50°, 3 h) polysaccharide (PS2) was typical of a regular polymer and contained signals for 4 anomeric carbons atoms at 99.8, 99.5, 99.0, and 97.6 p.p.m., 2 *C*-methyl groups of 6-deoxyhexoses at 17.9 and 17.4 p.p.m., 3 carbon atoms carrying nitrogen at 53.4, 50.8, and 49.3 p.p.m., 13 carbon atoms carrying oxygen in the region 67–80 p.p.m., 2 acetamido methyl groups at 23.4 and 23.1 p.p.m., 4 carbonyl groups in the region 174–176 p.p.m., and 1 formyl group at 166.0 p.p.m. (doublet in the gated-decoupling spectrum, J_{CH} 195 Hz); signals for hydroxymethyl groups v absent! Therefore. it was proposed that the tetrasaccharide repeating-unit of PS2 comprised two 6-deoxyhexoses and two uronic acid derivatives, three of the monosaccharides being amino sugars with two *N*-acetylated and one *N*-formylated.

Hydrolysis (2M HCl, 100°, 4 h) or solvolysis (HF, 20°, 3 h) of PS2 followed by conventional sugar analysis resulted in the identification of L-rhamnose (20%) and D-glucose (6%); the latter sugar is present in the core region of the *P. aeruginosa* lipopolysaccharides[7]. Amino sugars were detected only in trace amounts. Further, when PS2 was carboxyl-reduced[8], only one of two uronic acid derivatives was converted into hexose, as concluded from the appearance in the ^{13}C-n.m.r. spectrum of a signal for a hydroxymethyl group at 61.9 p.p.m. with intensity equal to unity. Solvolysis of the reduced polysaccharide (PS3) with HF gave L-rhamnose, 2-acetamido-2,6-dideoxy-D-glucose, and 2-acetamido-2-deoxy-D-galacturonamide (1), which were separated by preparative p.c.

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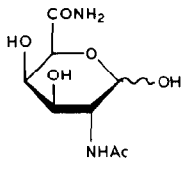
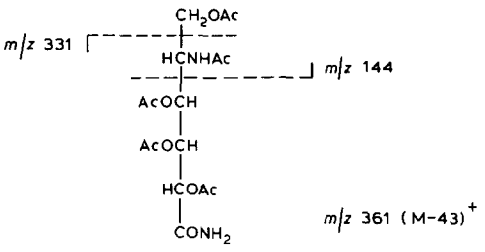
The structure of **1** was deduced from the mass spectrum of the derivative (**2**) obtained from **1** by borohydride reduction followed by acetylation. The ^1H coupling constants ($J_{2,3}$ 11, $J_{3,4}$ 3, and $J_{4,5}$ 1.5 Hz) for **1** together with an $[\alpha]_D$ value of +17° (water) {*cf.* $[\alpha]_D$ +29° (water) for 2-acetamido-2-deoxy-D-galacturonic acid[9]} were indicative of the D-*galacto* configuration.



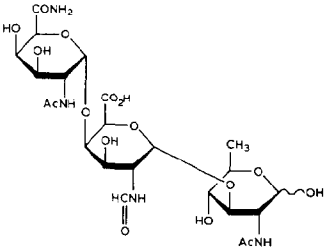
1



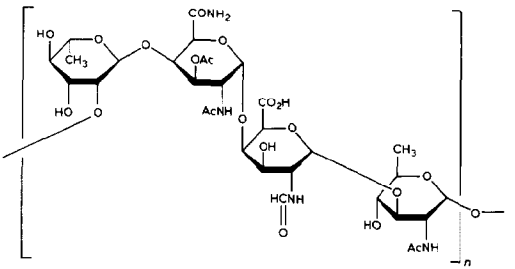
2

When PS3 was *N*-deformylated (0.05M HCl, 100°, 1 h) and then *N*-acetylated[10], it gave PS4 containing three *N*-acetyl groups per repeating-unit. Solvolysis of PS4 with HF yielded the afore-mentioned sugars as well as 2-acetamido-2-deoxy-D-galactose (derived from 2-deoxy-2-formamido-D-galacturonic acid). The same two new derivatives of 2-amino-2-deoxy-D-galacturonic acid were also detected by us in *P. aeruginosa* 0:4 (Lanyi classification[11]) lipopolysaccharides.

Recently[6], the O-specific polysaccharide of *P. aeruginosa* immunotype 1 was suggested to be composed of trisaccharide repeating-units made up of rhamnose, glucose, and 2-acetamido-2,6-dideoxyglucose together with *O*-acetyl and *O*-formyl subsituents. These data conflict with the ^{13}C-n.m.r. data for the polysaccharide and its monosaccharide composition described above. The revised structure (**4**) of the polysaccharide was established as follows.



3



4

PS2 was solvolysed with HF (20°, 1.5 h) to give oligosaccharide 3, which was isolated by gel filtration on Sephadex G-15. The presence in the ^{13}C-n.m.r. spectrum of 3 of two series of signals for α and β forms of reducing 2-acetamido-2,6-dideoxyglucose as well as slight splitting or broadening of the signals for the penultimate 2-deoxy-2-formamidogalacturonic acid residue allowed determination of the sequence of the amino sugar residues, which was supported by the result of borohydride reduction of **3**. Comparison of the ^{13}C-n.m.r. spectra of PS2 and 3 together with methylation analysis[12] data for PS4

revealed rhamnose to be substituted at O-2, 2-acetamido-2,6-dideoxyglucose at O-3, and both galactosaminuronic acid derivatives at O-4, thus showing the polysaccharide to be unbranched. The relatively large $^1J_{CH}$ values (170–172 Hz) determined from the gated-decoupling spectrum of PS2 for all of the anomeric carbon atoms indicated that the four hexopyranosyl residues were α-linked[13]. Finally, comparison of the ^{13}C-n.m.r. data for PS1 and PS2 indicated the *O*-acetyl groups to be located at position 3 of the 2-acetamido-2-deoxygalacturonamide residues and the extent of *O*-acetylation to be ~80%. The full interpretations of the ^{13}C-n.m.r. spectra of PS1–PS4 were in agreement with the proposed structure of the repeating unit for the polysaccharide.

REFERENCES

1 Yu. A. Knirel, N. A. Kocharova, A. S. Shashkov, B. A. Dmitriev, and N. K. Kochetkov, *Carbohydr. Res.*, 93 (1981) C12–C13.
2 Yu. A. Knirel, E. V. Vinogradov, A. S. Shashkov, B. A. Dmitriev, and N. K. Kochetkov, *Carbohydr. Res.*, 104 (1982) C4–C7.
3 Yu. A. Knirel, E. V. Vinogradov, A. S. Shashkov, B. A. Dmitriev, and N. K. Kochetkov, *Carbohydr. Res.*, 112 (1983) C4–C6.
4 Yu. A. Knirel, E. V. Vinogradov, V. L. L'vov, N. A. Kocharova, A. S. Shashkov, B. A. Dmitriev, and N. K. Kochetkov, *Carbohydr. Res.*, 133 (1984) C5–C8
5 O. Westphal and K. Jann, *Methods Carbohydr. Chem.*, 5 (1965) 83–91.
6 D. Horton, D. A. Riley, S. Samreth, and M. G. Schweitzer, in L. Anderson and F. M. Unger (Eds.), *Bacterial Lipopolysaccharides*, American Chemical Society, Washington, 1983, pp. 21–47.
7 P. S. N. Rowe and P. M. Medow, *Eur. J. Biochem.*, 132 (1983) 329–337.
8 R. L. Taylor, J. E. Shively, and H. E. Conrad, *Methods Carbohydr. Chem.*, 7 (1976) 141–151.
9 E. Darakas, H. Hultberg, K. Leontein, and J. Lönngren, *Carbohydr. Res.*, 103 (1982) 176–180.
10 K. Nagasawa and Y. Inoue, *Methods Carbohydr. Chem.*, 8 (1980) 291–294.
11 B. Lanyi, *Acta Microbiol. Acad. Sci. Hung.*, 13 (1966–67) 295–318.
12 H. E. Conrad, *Methods Carbohydr. Chem.*, 6 (1972) 361–364.
13 K. Bock and C. Pedersen, *J. Chem. Soc., Perkin Trans. 2*, (1974) 293–297.