Carbohydrate Research, 170 (1987) c1–c4
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Preliminary communication

Determination of the degree of amidation of 2-deoxy-2-formamido-D-galacturonic acid in O-specific polysaccharides of *Pseudomonas aeruginosa* O4 and related strains

EVGENY V. VINOGRADOV, YURIY A. KNIREL*, ALEXANDER S. SHASHKOV, AND NIKOLAY K. KOCHETKOV
N. D. Zelinsky Institute of Organic Chemistry, Academy of Sciences of the U.S.S.R., Moscow (U.S.S.R.)
(Received June 26th, 1987; accepted for publication, August 10th, 1987)

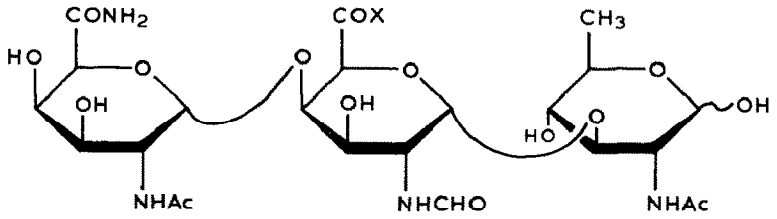
Reference strains of *P. aeruginosa* serogroup O4 (according to the Lányi classification, this includes three subgroups: O4a,4b, O4a,4c, and O4a,4d), serogroup O6 (Habs classification), group G (Homma classification), and immunotype 1 (Fisher classification) are serologically related[1], and produce structurally similar O-specific polysaccharides built up of tetrasaccharide repeating-units, containing 2-acetamido-2-deoxy-D-galacturonic acid, 2-deoxy-2-formamido-D-galacturonic acid, 2-acetamido-2,6-dideoxy-D-glucose, and L-rhamnose[2,3]. In each polysaccharide, the residues of 2-acetamido-2-deoxygalacturonic acid are present in the form of primary amide[2,3]; in the group G polysaccharide, half of the 2-deoxy-2-formamidogalacturonic acid residues are also amidated[3]. We now describe the determination of the degree of amidation of this sugar in the other polysaccharides.

The polysaccharide of each strain was treated with anhydrous hydrogen fluoride (3 h, 20°), and trisaccharides **1** and **2** thus obtained were reduced with sodium borohydride to give the oligosaccharide-alditols **3** and **4**. Acidic oligosaccharide **3** was isolated from the mixture derived from O4a,4d polysaccharide by anion-exchange h.p.l.c. (TSK-DEAE column; eluent, 0.03M sodium chloride in 0.01M sodium phosphate buffer, pH 6). Neutral oligosaccharide **4**, derived from immunotype 1 polysaccharide, was purified by reversed-phase h.p.l.c. (Alltech C18 column, water as eluent). The structure of **3** was established formerly[2] and was further confirmed by ^{1}H-n.m.r. spectroscopy, using sequential, selective spin-decoupling and nuclear Overhauser effect experiments. The same technique and ^{13}C-n.m.r. spectroscopy was applied to determine the structure of compound **4**.

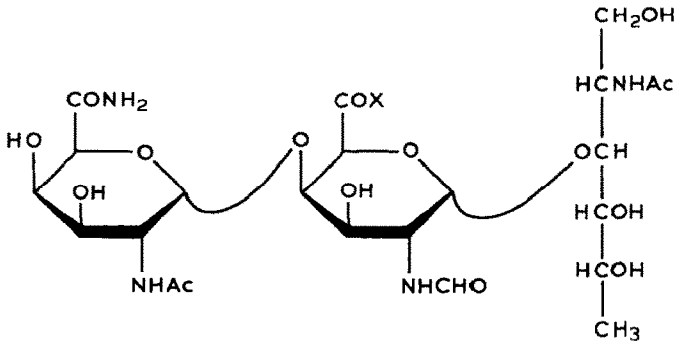
To prove the location of the free carboxyl group in **3** and its absence from **4**, the pH-dependence of their n.m.r. spectra in aqueous solutions was studied. The chemical shift of the signal for H-5 of 2-deoxy-2-formamidogalacturonic acid in the

*To whom correspondence should be addressed.

0008-6215/87/$ 03.50



1 X = OH
2 X = NH_2



3 X = OH
4 X = NH_2

TABLE I

CHEMICAL SHIFTS IN ^{13}C-N.M.R. SPECTRA[a] (δ, p.p.m.)

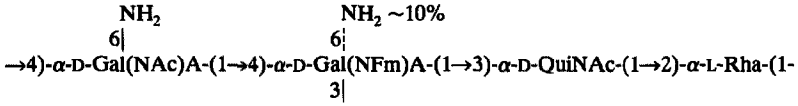
Compound and conditions	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
	2-Acetamido-2-deoxy-D-galacturonic acid residue					
Oligosaccharide **3**, pH 9	100.0	50.5	68.7	70.0	72.7	
Oligosaccharide **3**, pH 1	99.7	50.6	68.4	70.0	72.8	
Oligosaccharide **4**	99.8	50.5	68.3	70.0	72.6	
	2-Deoxy-2-formamido-D-galacturonic acid residue					
Oligosaccharide **3**, pH 9	99.4	49.3	67.5	79.6	72.7	
Oligosaccharide **3**, pH 1	99.6	49.3	67.5	78.4	71.6	
Oligosaccharide **4**	99.0	49.3	67.7	76.5	72.1	
	2-Acetamido-2,6-dideoxyglucitol residue					
Oligosaccharide **3**, pH 9	62.1	54.6	76.1	77.4	67.9	19.7
Oligosaccharide **3**, pH 1	62.0	54.5	75.9	77.8	67.9	19.5
Oligosaccharide **4**	61.7	54.7	75.6	77.7	67.9	19.6

[a]Recorded at 75 MHz for solutions in D_2O at 40° using acetone as internal standard (31.45 p.p.m. from tetramethylsilane). Chemical shifts for CH_3CO and COOH, 174–176 p.p.m.; for CH_3CO, 23–24 p.p.m. pH Values were adjusted with NaOD or CF_3COOD.

^{1}H-n.m.r. spectrum of **3** depends significantly on pH, changing from 4.26 p.p.m. at pH 9 to 4.60 p.p.m. at pH 1; similar displacement by +0.4 p.p.m. is observed for the signal for H-5 of galacturonic acid on acidification. The chemical shifts of the signals for C-4 and C-5 of this residue in the ^{13}C-n.m.r. spectrum of **3** (Table I) also show the pH-dependence that is characteristic for uronic acids[4]. In contrast, neither the signal for H-5 (4.86 p.p.m.) nor those for C-4,5 of the 2-acetamido-2-deoxygalacturonic acid residue change their position with the change of pH, thus confirming the carboxyl group of this residue to be amidated. Practically no pH-dependence was observed for the ^{1}H- and ^{13}C-n.m.r. spectra of **4**, and, hence, both uronic acid residues in this oligosaccharide are amidated. These conclusions were confirmed by fast-atom-bombardment mass-spectrometric determination of molecular weights of **3** and **4** (627 and 626, respectively), which are consistent with the structures of these compounds as monoamide and diamide, respectively.

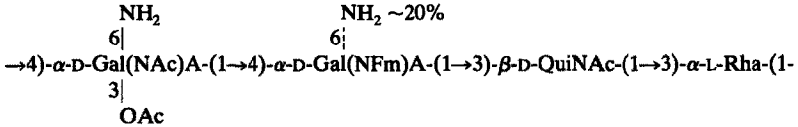
The degree of amidation of 2-deoxy-2-formamidogalacturonic acid was estimated as the ratio of oligosaccharides **4** and **3** in the mixtures derived from each polysaccharide. The ratio was measured by anion-exchange and reversed-phase h.p.l.c. (see above), monitored by u.v. absorbance at 220 nm. In the immunotype 1 polysaccharide, each residue of this uronic acid was amidated, whereas, in subgroup O4a,4c and serogroup O6 polysaccharides, each of these residues has a free carboxyl group. The subgroup O4a,4b and O4a,4d polysaccharides have 10% and 20%, respectively, of their 2-deoxy-2-formamidogalacturonic acid residues amidated. As judged by anion-exchange analysis, the last two polymers contain no neutral fraction, and, hence, both acidic monoamidated and neutral diamidated tetrasaccharide units are present in the same chain.

On the basis of the data reported here and elsewhere[2], it was concluded that the polysaccharides studied have the following structures:

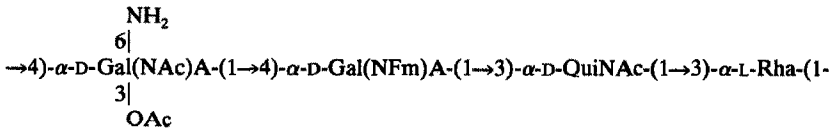


subgroup O4a,4b

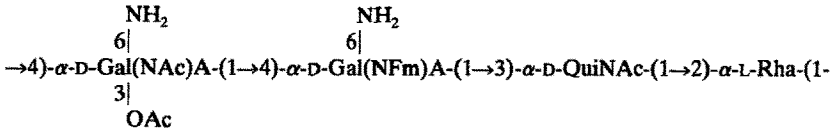
subgroup O4a,4c



subgroup O4a,4d



serogroup O6



immunotype 1

where Gal(NAc)A and Gal(NFm)A are 2-acetamido-2-deoxy- and 2-deoxy-2-formamido-galacturonic acids, QuiNAc is 2-acetamido-2,6-dideoxyglucose, and NH_2 indicates the amidation of carboxyl group.

The group G polysaccharide[3] differs from the subgroup O4a,4c polysaccharide by the amidation of 50% of the residues of 2-deoxy-2-formamidogalacturonic acid.

The possible role of the amidation is to adjust the optimal acidity of the lipopolysaccharide layer, and it could be expected that the degree of amidation varies not only from strain to strain, but also depends on growing conditions.

REFERENCES

1 B. LÁNYI AND T. BERGAN, *Methods Microbiol.*, 10 (1978) 93–168.
2 Y. A. KNIREL, E. V. VINOGRADOV, A. S. SHASHKOV, B. A. DMITRIEV, N. K. KOCHETKOV, E. S. STANISLAVSKY, AND G. M. MASHILOVA, *Eur. J. Biochem.*, 150 (1985) 541–550.
3 S. YOKOTA, S. KAYA, T. KAWAMURA, Y. ARAKI, AND E. ITO, *J. Biochem. (Tokyo)*, 99 (1986) 1551–1561.
4 W. EGAN, *Magn. Reson. Biol.*, 1 (1980) 197–258.