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Note

Absence of *O*-formyl groups in *Klebsiella* polysaccharides

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Non-carbohydrate substituents, for example, ether, acetal, *N*-acyl, and *O*-acyl groups, are present in several polysaccharides. Previously, such groups could be overlooked in structural analyses, but nowadays, when ^{1}H- and ^{13}C-n.m.r. spectroscopy are used routinely in such studies, this should no longer happen. *O*-Formyl groups have been reported to be present in the extracellular polysaccharides elaborated by *Klebsiella* types K2[1], K54[2], and K63[3]. An ester of formic acid should give signals at δ ~8 in the ^{1}H-n.m.r. spectrum[4] and at δ ~160 in the ^{13}C-n.m.r. spectrum[5]. In the reported[3] spectra of K63, deacetylated K54, and oligosaccharides[6] prepared by enzymic hydrolysis of K54, no such signals were observed, but signals at δ 4.7 (K54) and ~5.9 (K63) were assigned to the proton of the *O*-formyl group and signals at δ ~174 to the carbon atom of this group. The former signals are in the region for anomeric protons and methine protons on acyloxylated carbons, and the latter are in the region for carbonyl carbons of carboxylic acids other than formic acid and their esters. In making these assignments, it was necessary to assume that the *O*-formyl groups of partially formylated polyhydroxy compounds (in solution in D_2O) give signals at totally unexpected fields. If this were correct, *O*-formyl groups could have been overlooked in other n.m.r. studies of polysaccharides, and we have therefore investigated this matter more closely.

N.m.r. spectra of native K54 and K2 polysaccharides did not show any signals

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in the regions reported as typical of *O*-formyl groups, namely, δ ~8 (^{1}H) and ~160 (^{13}C).

Methyl 2-*O*-formyl-α-D-glucopyranoside (**1**) and the corresponding 3-*O*-formyl derivative (**2**) were prepared by formylation of methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside[7] and the corresponding 2-*O*-benzyl derivative[7], followed by deprotection. In the ^{13}C-n.m.r. spectra of these glycosides, the signals for the carboxyl groups appeared at δ ~165, but all other signals were close to those given by the corresponding *O*-acetyl derivatives[8]. In the ^{1}H-n.m.r. spectra, the signal for the formyl proton appeared at δ 8.23 (**1**) and 8.32 (**2**), respectively. The signals for H-1 and H-2 in **1** appeared at δ 5.00 ($J_{1,2}$ 3.5 Hz) and 4.83 ($J_{1,2}$ 3.5, $J_{2,3}$ 10 Hz), and those for H-1 and H-3 of **2** at δ 4.86 ($J_{1,2}$ 3.5 Hz) and 5.07 ($J_{2,3}$ 9, $J_{3,4}$ 9 Hz), respectively. Thus, the *O*-formyl groups in these glucosides give the expected n.m.r. signals, and there is reason to believe that *O*-formyl groups in polysaccharides should behave similarly.

The amorphous formates **1** and **2** were labile and showed rapid acyl migration as evident from their n.m.r. spectra. These spectra were therefore determined immediately after the deprotection of the corresponding *O*-benzylated 4,6-*O*-benzylidene derivatives.

Polysaccharide K54 is composed of tetrasaccharide repeating-units, and it has been reported that every repeating-unit contains an *O*-formyl group and that every second repeating-unit contains an *O*-acetyl group. On enzymic degradation[9] of K54 by treatment with the host bacteriophage Ø54, two tetrasaccharides (**3** and **4**) are therefore formed, one with, and one without, an *O*-acetyl group. Both should contain an *O*-formyl group (not represented in the formulas).

α-D-Glc*p*A-(1→3)-α-L-Fuc*p*-(1→3)-D-Glc*p*

2 4

| ↑

R 1

β-D-Glc*p*

3 R = OH

4 R = OAc

The molecular weights of **3** and **4**, assuming that each contains an *O*-formyl group, are 692 and 734, respectively; without the *O*-formyl group, the values are 664 and 706, respectively. Fast-atom bombardment (f.a.b.) mass spectra of **3**, in glycerol or glycerol–thioglycerol, showed an $[M + H]^+$ ion at *m/z* 665 and an $[M - H]^-$ ion at *m/z* 663. The corresponding values for **4** were *m/z* 707 and 705. No signals were present at the masses expected for the formylated compounds. These results therefore conclusively demonstrate that **3** and **4** do not contain *O*-formyl groups.

From the combined evidence given above, it is evident that K54 does not contain *O*-formyl groups and it seems likely that K2 and K63 do not contain such

groups either. Formic acid is formed when reducing sugars are treated with acids or bases, and the formic acid observed on analysis of these polysaccharides is probably an artefact.

EXPERIMENTAL

General methods. — Melting points are corrected. Concentrations were performed under diminished pressure at <40° (bath). Optical rotations were measured at 22° with a Perkin–Elmer 241 polarimeter. For n.m.r. spectroscopy, a JEOL FX-100 or GX-400 spectrometer was used. Chemical shifts are reported in p.p.m. downfield from internal Me_4Si (^1H and ^{13}C, solutions in $CDCl_3$), downfield from external Me_4Si (^{13}C), or relative to internal acetone δ 2.23 downfield from internal sodium 1,1,2,2,3,3-hexadeuterio-4,4-dimethyl-4-silapentane-1-sulfonate (^1H, solutions in D_2O). All spectra were obtained at ambient temperature except for the ^1H-n.m.r. spectrum of **1**, which was recorded at 50° in order to remove the water peak from the signal of H-2. Preparative separations were performed on columns (4 × 35 cm) of Kieselgel 60 (0.040–0.063 mm, Merck).

F.a.b.-m.s. was carried out with a VG Analytical ZAB-HF mass spectrometer. Xenon was used as the bombarding gas and the atom gun was operated at 8 kV. Samples were dissolved in aqueous 5% acetic acid (1–5 μg/μL), and 1 μL was loaded into a drop of glycerol on the stainless-steel target. After positive and negative ion spectra had been acquired, 0.5 μL of thioglycerol was added and further spectra were obtained. The thioglycerol suppressed the glycerol cluster-ions so that any sample ions occurring at the same masses as the cluster ions could be observed.

Methyl 3-O-benzyl-4,6-O-benzylidene-2-O-formyl-α-D-glucopyranoside. — To a solution of methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside[7] (372 mg) in ether (1 mL) and *N*,*N*-dimethylformamide (100 μL) was added acetic formic anhydride[10] (1 mL), and the mixture was stirred at room temperature for 6 days and then concentrated. The resulting syrup was eluted from a column of silica gel with toluene–ethyl acetate (2:1) to yield the title compound (305 mg, 76%), m.p. 82–83°, $[\alpha]_{578}^{22}$ +57° (*c* 1, chloroform). N.m.r. data ($CDCl_3$): ^{13}C, δ 55.2, 62.3, 68.7, 72.5, 74.7, 75.8, 81.9, 97.6, 101.3, 126.0, 127.7, 128.2, 128.9, 137.3, 138.4, and 160.0; ^1H, 8.08 (s, -OC*H*O).

Anal. Calc. for $C_{22}H_{24}O_7$: C, 66.0; H, 6.04. Found: C, 66.3; H, 6.01.

Methyl 2-O-formyl-α-D-glucopyranoside (**1**). — Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-formyl-α-D-glucopyranoside (64 mg) was dissolved in acetic acid (5 mL) and hydrogenated at 1 atm. over 10% Pd/C (150 mg) overnight. The solution was filtered and concentrated, to yield **1** (26 mg, 76%) as a syrup. N.m.r. data (D_2O): ^{13}C, δ 163.8 (-OC*H*O), 97.7 (C-1), 73.9 (C-2), 72.8 (C-5), 71.8 (C-3), 70.6 (C-4), 61.6 (C-6), and 55.9 (OMe); ^1H, δ 8.23 (s, -OC*H*O), 5.00 ($J_{1,2}$ 3.5 Hz, H-1), and 4.83 ($J_{1,2}$ 3.5, $J_{2,3}$ 10 Hz, H-2).

Methyl 2-O-benzyl-4,6-O-benzylidene-3-O-formyl-α-D-glucopyranoside. — Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (186 mg) was con-

verted into the title compound (140 mg, 70%), m.p. 102–103°, $[\alpha]_{578}^{22}$ +26° (*c* 1, chloroform), essentially as described for the 2-*O*-formyl derivative. N.m.r. data ($CDCl_3$): ^{13}C, δ 55.4, 62.2, 68.8, 72.0, 73.1, 77.3, 78.9, 98.5, 101.4, 126.1, 127.9, 128.2, 128.5, 129.0, 136.9, 137.5, and 160.8; ^1H, δ 8.14 (s, -OC*H*O).

Anal. Calc. for $C_{22}H_{24}O_7$: C, 66.0; H, 6.04. Found: C, 65.5; H, 6.06.

Methyl 3-O-formyl-α-D-glucopyranoside (**2**). — Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-formyl-α-D-glucopyranoside (115 mg) was hydrogenated, as described above for the preparation of **1**, to yield **2** (41 mg, 69%) as a syrup. N.m.r. data (D_2O): ^{13}C, δ 165.2 (-OC*H*O), 100.2 (C-1), 77.6 (C-3), 72.5 (C-5), 70.3 (C-2), 68.5 (C-4), 61.5 (C-6), and 56.3 (OMe); ^1H, δ 8.32 (s, -OC*H*O), 4.86 ($J_{1,2}$ 3.5 Hz, H-1), and 5.07 ($J_{2,3}$ 9, $J_{3,4}$ 9 Hz, H-2).

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