Carbohydrate Research, 63 (1978) 323–326

Note

Presence of D-galactofuranose in the capsular polysaccharide of *Klebsiella* serotype K-41: synthesis of 5,6-di-*O*-methyl-D-galactofuranose

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(Received May 3rd, 1977; accepted for publication in revised form, August 30th, 1977)

Capsular polysaccharides of *Klebsiella* show a great diversity[1,2] both in their sugar composition and in the regular structure of their repeating units. Among the structures thus far reported for the K-antigen from *Klebsiella*, the polysaccharide from serotype K41 is particular[3] in that its repeating unit consists of seven sugar residues: rhamnose, glucose, galactose, and glucuronic acid in the relative proportions 1:3:2:1. Another individual feature is that the repeating unit has a trisaccharide side-chain attached to position 3 of a galactofuranose residue. This is the first example of the presence of a galactofuranose residue in the K-antigen of *Klebsiella*. Galactofuranose has been identified in the lipopolysaccharides of *Klebsiella* O groups[4,5] 8 and 9, where it is linked to position 3 in the chain.

Isolation of 5,6-di-*O*-methylgalactose from the hydrolysate of the permethylated K-41 polysaccharide demonstrated the presence of a 2,3-linked galactofuranose residue. This di-*O*-methyl derivative was identified by g.l.c.–m.s. of its alditol acetate, which showed characteristic ions in the m.s.: *m/e* 45, 89, and 333.

The retention time in g.l.c. of 1,2,3,4-tetra-*O*-acetyl-5,6-di-*O*-methyl-D-galactitol (R_T 3.22 on an ECNSS-M column at 155° relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol) corresponded exactly with that of the authentic compound whose synthesis is reported here. Further evidence of the presence of a 2,3-linked galactofuranose residue in K-41 was provided by the periodate oxidation–sodium borohydride reduction–hydrolysis sequence, which afforded L-arabinofuranose among the hydrolysis products. The presence of the latter is compatible only with the existence in the original polymer of D-galactofuranosyl groups having two free hydroxyl groups at positions 5 and 6. To our knowledge, the synthesis of 5,6-di-*O*-methyl-D-galactofuranose has not yet been reported.

Treatment[6] of methyl β-D-galactofuranoside[7] (**1**) with acetone gave the 5,6-isopropylidene acetal (**2**) in 95% yield. Benzylation[6] of **2** gave methyl 2,3-di-*O*-benzyl-5,6-*O*-isopropylidene-β-D-galactofuranoside (**3**) in 65% yield. Cleavage of the isopropylidene group at room temperature with dilute acetic acid[8] yielded 71.5% of **4**. This product was methylated according to Hakomori[9] to give the corresponding

5,6-di-methyl ether **5** in 44% yield. Hydrogenolysis of **5** yielded 77% of methyl 5,6-di-*O*-methyl-β-D-galactofuranoside (**6**) which, on hydrolysis[10], gave 5,6-di-*O*-methyl-α-D-galactofuranose (**7**). Reduction and acetylation[11] of the latter allowed characterization of 1,2,3,4-tetra-*O*-acetyl-5,6-di-*O*-methyl-D-galactitol (**8**) *in* g.l.c.–m.s.

The behaviour of compound **8** in g.l.c., and its fragmentation in m.s., thus confirmed the identification of the presence of the 2,3-linked galactofuranose in the original polysaccharide K-41.

The methyl α- and β-D-galactofuranosides[7] served as model compounds for assignment of the n.m.r. spectra obtained with the original[3] K-41. Application of the INDOR technique to the ^{1}H-n.m.r. spectrum of methyl β-D-galactofuranoside gave the following results*: δ 4.46 (d, $J_{1,2}$ 1.5 Hz, H-1), 3.56–3.66 (m, H-2 and H-3), 3.50 (q, $J_{3,4}$ 4 Hz, $J_{4,5}$ 5.6 Hz, H-4), 3.37 (m, H-5), 3.1–3.3 (m, H-6a,6b), 2.98 (OCH_3). The ^{13}C-n.m.r. data obtained for the methyl α- and β-D-galactofuranosides are in good agreement with those found by Gorin and Mazurek[12], and showed characteristic coupling constants of $^1J_{C-1,H-1}$ of 172.5 Hz for the β anomer and $^1J_{C-1,H-1}$ of ~175 Hz for the α anomer. Similarly, the intermediate compound **4** served as a model for the 2,3-di-*O*-substituted galactofuranose found in K-41. The n.m.r. data for **4** are given in the experimental section.

EXPERIMENTAL

Analytical methods used on the K-41 polysaccharide will be reported separately[3].

General methods. — Melting points were determined using a Büchi apparatus and are uncorrected. Optical rotations were measured with a Roussel et Jouan Quick polarimeter at 20°. G.l.c. was performed on a Packard–Becker instrument Chromatograph Model 407 equipped with column A (200 × 0.4 cm) of 3% ECNSS-M on Gas-Chrom Q, or on column B (200 × 0.3 cm) of 10% DEGS on Gas-chrom W. N.m.r. spectra were recorded on a Cameca-250 spectrometer, with deuterium oxide or chloroform-*d* as solvent.

Methyl β-D-galactofuranoside (**1**). — This compound was prepared according to Augestad and Berner[7], in 56% yield; m.p. 66°, $[\alpha]_D$ −143° (*c* 3.2, methanol), *lit.*[7] m.p. 69°, $[\alpha]_D$ −140° (*c* 3, methanol).

Anal. Calc. for $C_7H_{14}O_6$: C, 43.33; H, 7.21. Found: C, 43.33; H, 7.04.

Methyl 5,6-O-isopropylidene-β-D-galactofuranoside (**2**). — To a solution (250 ml) of acetone containing sulfuric acid (0.2 ml), 3.98 g (20.53 mmol) of **1** and 12 g of anhydrous copper sulfate were added. The mixture was stirred for 8 h at room temperature[6]. After neutralisation and filtration, the solution of product was purified

*The spectrum was recorded in D_2O with tetramethylsilane as external reference in a capillary tube. In order to express these chemical shifts in reference to T.S.P. (sodium 2,2,3,3-tetradeuterio-4,4-dimethyl-4-silapentanoate), +0.8 p.p.m. must be added.

on a column of silica gel eluted with butanone, affording **2** (4.55 g, 19.44 mmol, 95% yield): b.p. 120°/0.25 mm, $[\alpha]_D$ −81° (*c* 2.6, chloroform).

Anal. Calc. for $C_{10}H_{18}O_6$: C, 51.31; H, 7.69. Found: C, 51.49; H, 7.77.

*Methyl 2,3-di-*O*-benzyl-5,6-*O*-isopropylidene-β-*D*-galactofuranoside* (**3**). — To a solution of **2** (3.65 g) in dry dimethyl sulphoxide (50 ml) was added methylsulphinyl anion (2M, 20 ml) at 20°. α-Chlorotoluene (7.5 ml) in dimethyl sulphoxide (15 ml) was then added dropwise. After stirring for one h, benzene (250 ml) was added to the mixture. The benzene extract was washed with saturated sodium hydrogencarbonate and then water, and dried with sodium sulphate. Evaporation gave a syrup that was purified on a column of silica gel eluted with 3:1 dichloromethane–ethyl ether yielding **3** (4.10 g; 63.5%), b.p. 180°/0.25 mm, $[\alpha]_D$ −55° (*c* 2.7, chloroform).

Anal. Calc. for $C_{24}H_{30}O_6$: C, 69.56; H, 7.24. Found: C, 69.18; H, 6.97.

*Methyl 2,3-di-*O*-benzyl-β-*D*-galactofuranoside* (**4**). — Cleavage of the isopropylidene group in **3** (1.83 g) was effected with 7:3 (v/v) acetic acid–water (65 ml) for 24 h at room temperature[8] to give **4**, which crystallized from ethyl ether in 71.5% yield, homogeneous on silica gel t.l.c. (3:1 dichloromethane–ethyl ether, R_F 0.14; butanone, R_F 0.74); m.p. 91°, $[\alpha]_D$ −80° (*c* 3.0 chloroform); ^{1}H-n.m.r. ($CDCl_3$ + Me_4Si): δ 7.5–7.2, (aromatic), 4.9 (H-1), 4.6–4.4 (2-CH_2, benzyl), 4.14–3.30 (H-2,3, and 4), 3.85–3.50 (H-5,6a, and 6b), 3.34 (OCH_3), 3.2–2.7 (OH-5, OH-6); ^{13}C-n.m.r. ($CDCl_3$ + Me_4Si): δ 107.40 (C-1), 87.40, 83.40, and 82.40 (C-2, C-3, and C-4, not individually assigned), 72.40, 71.95 (CH_2 of benzyl*), 71.50 (C-5*), 64.40 (C-6), and 54.85 (OCH_3).

*Methyl 2,3-di-*O*-benzyl-5,6-di-*O*-methyl-β-*D*-galactofuranoside* (**5**). — Methylation of **4** (0.6 g) was performed according to Hakomori[9]. Extraction with chloroform and purification on a column of silica gel eluted with 3:1 dichloromethane–ethyl ether gave **5** (44% yield), b.p. 204°/0.8 mm), $[\alpha]_D$ −64° (*c* 1.7, chloroform).

Anal. Calc. for $C_{23}H_{30}O_6$: C, 68.65; H, 7.46. Found: C, 68.39; H, 7.53.

*Methyl 5,6-di-*O*-methyl-β-*D*-galactofuranoside* (**6**). — Hydrogenolysis of **5** (0.23 g) in ethanol (30 ml) was effected with palladium on charcoal and afforded **6** (98 mg, 77%), homogeneous on t.l.c. (silica gel 3:1 dichloromethane–ether, R_F 0.1; butanone, R_F 0.52); $[\alpha]_D$ −107° (*c* 2.7 chloroform). It gave a single peak in gas chromatography on column B at 180° (R_T 7.1 relative to methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranoside).

*5,6-Di-*O*-methyl-α-*D*-galactofuranose* (**7**). — Compound **6** was hydrolysed according to Robertson and Lamb[10] and gave a colorless syrup, homogeneous by chromatography on Whatman no. 1 paper (4:1:4.9:0.1 1-butanol–ethanol–water–ammonium hydroxide, R_T 0.53; R_G 0.65 relative to 2,3,4,6-tetra-*O*-methyl-D-glucose); b.p. 140°/0.3 mm, $[\alpha]_D^{20}$ −20° (10 min) → 0° (equil.) (*c* 1, water).

*1,2,3,4-Tetra-*O*-acetyl-5,6-di-*O*-methyl-*D*-galactitol* (**8**). — Reduction of **7** with

*An off-resonance experiment allowed differentiation between C-5 and the two methylenic carbon atoms of the benzyl ethers.

sodium borohydride, followed by acetylation[11], gave the alditol acetate **8**, which wa examined directly by g.l.c. on column A at 155° and then by g.l.c.–m.s. on an OV-22 column at 150°. The product showed the expected fragments at *m/e* 43 (100), 45 (17) 59 (21.7), 89 (58.3), 97 (46.7) 129 (35.2), 139 (74.2), 149 (25.8), 171 (16.6), 259 (12.8) 333 (28.8).

ACKNOWLEDGMENTS

This work is part of a research program between the N.R.C. Canada (Professo G.G.S. Dutton, U.B.C., Vancouver) and the CNRS-France (CERMAV, Grenoble) The kind help of Dr. M. Vignon (CERMAV, Grenoble) with the n.m.r. spectroscop is gratefully acknowledged.

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