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Note

Structure of the capsular polysaccharide (K19 antigen) from uropathogenic *Escherichia coli* O25:K19:H12

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Uropathogenic *Escherichia coli* are usually encapsulated with acidic polysaccharides (K antigens[1,2]), many of which contain 3-deoxy-D-*manno*-2-octulosonic acid (KDO) as the acidic component, frequently together with one or two ribose moieties in the (di- or tri-saccharide) repeating unit[3–10]. The K13, K20, and K23 polysaccharides contain β-KDO*p*[5,7], the K6 polysaccharide α-KDO*p*[3], and the K74 and K95 polysaccharides β-KDO*f*[9,10]. Thus, KDO-ribose polysaccharides form a group of closely related, yet serologically distinct, capsular antigens, the variability being increased by different degrees of *O*-acetylation at various sites. We now report on the structure of the K19 antigen, another KDO-ribose-type polysaccharide.

The K19 polysaccharide, obtained[5,11] (92 mg/L) from liquid cultures of *E coli* E47a (O25:K19:H12) by precipitation with cetyltrimethylammonium bromide (Cetavlon), extraction with aqueous $CaCl_2$, precipitation with ethanol, and extraction with cold phenol (pH 6.8), had $[\alpha]_D^{25}$ +4.8° (*c* 0.1, water) and consisted of ribose, KDO, and OAc in the ratios 1:1:0.35.

Periodate oxidation, followed by borohydride reduction, converted ~40% of the KDO into a 3-deoxy-2-heptulosonic acid (p.e., M_{KDO} 1.1)[4], the ribose being unchanged. Periodate oxidation/borohydride reduction of the *O*-deacetylated K19 polysaccharide (K19d) converted all of the KDO into 3-deoxy-2-heptulosonic acid.

Methylation of the K19 polysaccharide (Hakomori[12,13]), followed by hydrolysis (aqueous 90% formic acid, 2 h, 100°; then with 0.125M sulfuric acid, 2 h, 100°), borohydride reduction, and acetylation, gave 1,3,4-tri-*O*-acetyl-2,5-di-*O*-methylribitol as determined by g.l.c. (ECNSS-M, 140°) and m.s. This finding indicated the presence of a 3-linked ribofuranoside moiety.

Hydrolysis (1% acetic acid, 30 min, 100°) of the K19 polysaccharide gave a disaccharide (**1**) which, on chromatography on Biogel P-2, had K_d 0.9 and, in paper

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electrophoresis (Schleicher and Schüll paper 2043a, pH 5.4, 42 V/cm, 90 min), had M_{KDO} 0.75. Methylation of **1**, using potassium methylsulfinylmethanide[14], gave a product, g.l.c. (SE 54, 5 min at 150°, then 5°/min to 300°) of which revealed four components each with a mol. wt. of 482 (c.i.-m.s.). An additional minor component with a mol. wt. of 436 was presumably a methylated disaccharide containing anhydro KDO[15] and was not studied further. The products with mol. wt. 482 had similar e.i.-mass spectra, indicative of methyl α- and β-glycosides of methylated **1** with either KDO*p* or KDO*f*. The fact that methylated **1** occurred with KDO*p* and KDO*f* indicated that, in **1** (and thus also in the K19 polysaccharide), either C-5 or C-6 of KDO is involved in ring formation. This inference, together with the fact that KDO in the polysaccharide could be oxidised with sodium metaperiodate between C-7 and C-8, indicated the KDO to be 4-linked.

The ^{13}C-n.m.r. spectra of the native (K19) and *O*-deacetylated (K19d) polysaccharides were compared with those of the *O*-deacetylated K13 polysaccharide [K13d; repeating unit, →3)-β-Rib-(1→7)-β-KDO*p*-(2→]$_5$ the *O*-deacetylated K95 polysaccharide [K95d; repeating unit, →3)-β-Rib-(1→8)-β-KDO*f*-(2→]$_{10}$, 2-*O*-Me-[β-Rib-(1→7)]-β-KDO*p* (**2**)[16], and 2-*O*-Me-[β-Rib-(1→7)]-α-KDO*p* (**3**)[16]. The signal assignments and the results of the attached proton test (APT)[17,18] with K19 and K19d are given in Table I. The latter spin technique differentiates between CH_3 and CH= (positive signals) and CH_2 and C≡ (negative signals). The signal at δ 33.1 was

TABLE I

^{13}C-N.M.R. DATA[a]

	Atom	*K19* δ *APT*[b]	*K19d* δ *APT*	*K13d* δ	*K95d* δ	**2** δ	**3** δ
KDO	C-1	174.2(−)	173.8(−)	174.2	176.5	174.4	176.1
	C-2	102.6(−)	102.4(−)	102.8	110.0	102.1	101.3
	C-3	33.1(−)	33.1(−)	35.6	45.1	35.3	35.0
	C-4	75.6(+)	75.8(+)	68.4	73.3	68.2	66.7
	C-5	65.8(+)	65.8(+)	66.4	87.2	66.0	66.8
	C-6	74.8(+)	74.9(+)	73.5	71.1	72.9	70.5
	C-7	69.9(+) 68.1(+)	70.1(+)	76.1	71.6	75.4	75.3
	C-8	65.1(−) 67.2(−)	65.1(−)	63.6	70.1	60.8	59.6
Ribose	C-1	106.8(+)	106.9(+)	105.7	108.6	105.8	106.0
	C-2	75.1(+)	75.2(+)	74.9	75.5	75.7	75.7
	C-3	74.4(+)	74.6(+)	74.9	75.5	71.2	71.0
	C-4	82.1(+)	82.2(+)	82.4	82.5	83.5	83.4
	C-5	62.6(−)	62.6(−)	61.1	63.9	63.2	62.7

[a]For the K19 polysaccharide before (K19) and after *O*-deacetylation [K19d, →3)-β-Rib*f*-(1→4)-β-KDO*p*-(2→], the *O*-deacetylated K13 polysaccharide [K13d, →3)-β-Rib-(1→7)-β-KDO*p*-(2→]$_5$, the *O*-deacetylated K95 polysaccharide [K95d, →3)-β-Rib-(1→8)-β-KDO*f*-(2→]$_{10}$, 2-*O*-Me-[β-Rib-(1→7)-β-KDO] (**2**)[16], and 2-*O*-Me-[β-Rib-(1→7)-α-KDO] (**3**). [b]The signs of the signals in the APT[17,18] are given in brackets.

due to C-3 of KDO*p*, in contrast with δ 45.1 for K95d (8-substituted KDO*f*) and δ 45.1 for K74 (6-substituted KDO*f*)[9], and was at lower field than those of K13d (δ 35.6), **2** (δ 35.3), **3** (δ 35.0), the K12 antigen (δ 36.2)[4], and the K14 antigen (δ 36.4)[6]. This indicated a β shift on C-3 due to substitution at C-4 of KDO, which was corroborated by an α shift of C-4 (δ 75.6 *vs.* 68.4 for K13d, δ 68.2 for **2**, and δ 66.7 for **3**).

The C-3 signal of ribose (δ 74.4 for K19, δ 74.6 for K19d), compared to δ 71.2 for **2** and δ 71.0 for **3**, indicated 3-substitution. The ^{13}C-n.m.r. data are in agreement with those of methylation.

The ^{13}C-n.m.r. spectrum of K19 contained two signals more (δ 68.1 and 67.2) than that of K19d. The signal at δ 67.2 (negative in APT) was assigned to C-8 of KDO with an α shift (2.1 p.p.m.) due to partial *O*-acetylation; likewise, the signal at δ 68.1 (positive in APT) was assigned to C-7 of KDO with a β shift (−1.1 p.p.m.). The OAc groups of the K19 polysaccharide (0.35 per repeating unit) are therefore located at C-8 of 35% of the KDO units.

The ^{1}H-n.m.r. spectrum of K19d indicated KDO to be β, as evidenced by the signals of H-3*a* (δ 2.56) and H-3*e* (δ 1.9)[19]. The β configuration was also evidenced by a positive Cotton effect ($mol^{-1}.cm^{-1}$)[20,21] at 217 nm (Δε +0.47), which agrees well with the value for 2-*O*-Me-β-KDO (Δε +0.13)[22] and for β-KDO in the K13 polysaccharide (Δε +0.49)[5] and is opposite to that for 2-*O*-Me-α-KDO (Δε −66)[22].

Based on the foregoing results, the structure of the K19 polysaccharide is proposed as →3)-β-Rib*f*-(1→4)-β-KDO*p*-(2→ with OAc groups at *C*-8 of about one-third of the KDO residues. This structure is similar to that [→3)-β-Rib*f*-(1→7)-β-KDO*p*-(2→] of the K13 polysaccharide[5], which has OAc groups at C-5 of about one-third of the KDO residues. Similar structural variations have been detected in about ten KDO-containing polysaccharides, and it would be of interest to translate the different primary structures into three-dimensional molecular structures.

EXPERIMENTAL

Bacteria and cultivation. — *E. coli* E74a (O25:K19:H12), obtained from Drs. I. and F. Ørskov (Copenhagen), was grown to the late logarithmic phase in a fermenter in 10-L batches, which contained per L: $K_2HPO_4 \cdot 3H_2O$ (9.7 g), K_2PO_4 (2 g), sodium citrate · $5H_2O$ (0.5 g), $MgSO_4 \cdot 7H_2O$ (0.1 g), casamino acids (20 g), ammonium acetate (20 g), D-glucose (2 g), and the dialysable part of yeast (100 mL from 500 g in 5 L of deionised water).

Isolation and purification of the capsular polysaccharide. — The acidic capsular polysaccharide together with the bacterial cells were precipitated from the liquid culture by the addition of 1 vol. of aqueous 2% cetyltrimethylammonium bromide (Cetavlon). All the following operations were carried out at 4°. The polysaccharide was extracted from the precipitate with M calcium chloride, and purified by three cycles of precipitation from aqueous solution with ethanol (80% final concentration) followed by repeated extractions with cold phenol (80%, buffered to pH

6.7 with sodium acetate)[5,11]. The combined aqueous phases were centrifuged for 4 h 105,000 *g* and the supernatant solution was lyophilised.

Analytical methods. — KDO was determined, after hydrolysis (0.1M trifluoroacetic acid, 10 min, 100°), by the thiobarbituric acid assay[23]. Ribose was determined, after hydrolysis (M H_2SO_4, 2 h, 100°), by g.l.c. of the alditol acetate on ECNSS-M and with the orcinol reagent. Acetate was determined by g.l.c. on Poropak QS[24] and also by ^1H- and ^{13}C-n.m.r. spectroscopy of the polysaccharide. High-voltage paper electrophoresis was run on Schleicher and Schüll 2043a paper (pH 5.3, 4.2 V/cm, 90 min). G.l.c. was performed with a Varian aerograph Series 1400 instrument, equipped with an autolinear temperature programmer and a Hewlett-Packard 3380 integrator, and g.l.c.-m.s. was performed with a Finnigan MAT 1020B automatic system at 70eV on a CB CP SIL 5 (25m × 0.25 mm) column, using helium as the carrier gas. C.i.-m.s. was done with ammonia as the reactant gas. N.m.r. spectra were recorded with a Bruker WM 300 spectrometer in the F.t. mode at 70° (^1H) and 33° (^{13}C) [external sodium 4,4-dimethyl-4-sila-(2,2,3,3-$^2H_4$)pentanoate]. The chemical shifts of the ^{13}C resonances were corrected (−1.31 p.p.m.) by using 1,4-dioxane (δ 67.4, based on Me_4Si). Optical rotations were measured with a Perkin-Elmer 141 polarimeter.

Periodate oxidation, reduction with sodium borohydride or sodium borodeuteride, *O*-deacetylation, as well as methylation[12,14] have been described[4,5,9,10].

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