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4-*O*-[(*S*)-1-CARBOXYETHYL]-D-GLUCURONIC ACID: A COMPONENT OF THE *Klebsiella* TYPE 37 CAPSULAR POLYSACCHARIDE*

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ABSTRACT

The acidic sugar component in the *Klebsiella* type 37 capsular polysaccharide (K 37) has been identified as 4-*O*-[(*S*)-1-carboxyethyl]-D-glucuronic acid. The identification is based upon chemical and spectroscopic studies, and the identity of the carboxyl-reduced sugar, 4-*O*-[(*S*)-2-(1-hydroxy)propyl]-D-glucose and derivatives, with the corresponding substances synthesized by an unambiguous route.

INTRODUCTION

The different capsular polysaccharides produced by *Klebsiella* species all contain acidic components[1,2]. In most of these polysaccharides, the acidic component is D-glucuronic acid or D-galacturonic acid, but the presence of unidentified acids in some polysaccharides was also demonstrated. One of these, in the type 38 polysaccharide, was identified as 3-deoxy-L-*glycero*-pentulosonic acid[3]. We now report studies on the acidic component in K 37.

RESULTS AND DISCUSSION

K 37 is composed of D-glucose, D-galactose, and an acidic sugar (*A*) which gave a positive reaction with carbazole[1]. On hydrolysis, carboxyl-reduced[4] K 37 yielded D-glucose, D-galactose, and a third component in the proportions 2:1:1, analysed by g.l.c. of their alditol acetates[5]. Methylation analysis of carboxyl-reduced K 37 yielded 2,3,4-tri-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-glucose, 2,6-di-*O*-methyl-D-galactose, and a fourth component in equimolar proportions. The fourth component consequently derives from a terminal sugar.

*Dedicated to the memory of Professor Edward J. Bourne.

The mixture of methylated sugars was analysed by g.l.c.–m.s.[6] of the derived alditol acetates. M.s. of the component derived from *A*, and of analogues prepared by performing the carboxyl-reduction and/or the reduction of sugar to alditol by deuterating reagents (Table I), furnished valuable structural information.

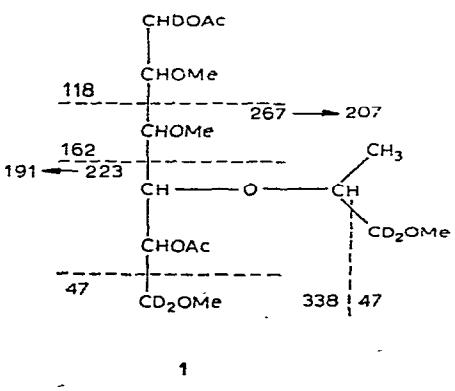
TABLE I

PERTINENT IONS IN THE MASS SPECTRA OF THE DERIVATIVES OF THE NEW SUGAR OBTAINED FROM THE CARBOXYL-REDUCED, FULLY METHYLATED K 37 ON ACID HYDROLYSIS, REDUCTION, AND ACETYLATION[a]

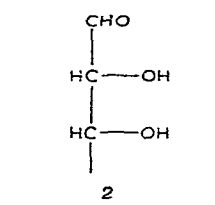
A m/e	B m/e	C m/e	D m/e
73	73	75	75
101	102	101	102
117	118	117	118
161	162	161	162
187	187	191	191
203	203	207	207
219	219	223	223
263	263	267	267
291	292	293	294
335	336	337	338

[a]Carboxyl-reduction and reduction of the sugar with the following sequences of reagents: *A*, BH_4^-, BH_4^-; *B*, BH_4^-,BD_4^-; *C*, BD_4^-,BH_4^-; *D*, BD_4^-,BD_4^-.

The structure deduced for the fully deuterated analogue **1**, omitting stereochemistry, is depicted below, and the origins of some pertinent m.s. fragments are indicated.



1



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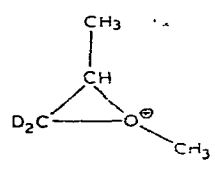


3

The primary fragments of *m/e* 117 and 161, shifting to 118 and 162 on reduction of the methylated sugar with borodeuteride, demonstrate the sequence **2** in the sugar.

The peaks of *m/e* 117 and 263, 161 and 219, and 45 and 335 from the undeuterated substance strongly indicate that the molecular weight is 380. The shift of some fragments by 4 mass units after carboxyl-reduction with deuteride, *e.g.*, *m/e* 219 to 223, demonstrates the presence of two carboxyl groups in the original acidic sugar. Further application of the principles outlined for the fragmentation of partially methylated alditol acetates[6] supports structure **1** (pentadeuterated). The elimination of acetic acid from *m/e* 267 and of methanol from *m/e* 223, both most probably by β-elimination, give the substituents at C-5 and C-6. The formation of a strong fragment M−47 shows that the carbon atom adjacent to one of the methoxymethyl groups is not acetoxylated but etherified, as in **1**. A strong fragment *m/e* 73, shifting to *m/e* 75 after carboxyl-reduction with borodeuteride, probably has structure **3** and derives from the substituent at O-4.

These results suggest that the acidic sugar is a hexuronic acid etherified with lactic acid at O-4.

In order to further elucidate the structure of *A*, the corresponding carboxyl-reduced compound *R* was isolated by preparative paper-chromatography of hydrolysates of reduced K 37. The results obtained by m.s. of the partially methylated alditol acetates were corroborated by investigation of the m.s. from the peracetylated α-anomer of *R* and of the permethylated methyl α-glycoside of *R*. Established fragmentation pathways proved to be useful in these investigations[7,8]. *R* had a low electrophoretic mobility in germanate buffer as expected for a 4-*O*-substituted glucose[9].

The alditol of *R*, on treatment with boron tribromide[10], yielded glucitol, identified by g.l.c.–m.s. of its acetate. Consequently, *A* has the *gluco* or *gulo* configuration.

Unambiguous evidence for the *gluco* configuration of *A* was obtained by investigation of the ^{1}H-n.m.r. spectrum of the α anomer of peracetylated *R*: H-1 appeared at δ 6.2 ($J_{1,2}$ 4 Hz), H-2 at δ 4.9 ($J_{1,2}$ 4 Hz, $J_{2,3}$ 10 Hz), and H-3 at δ 5.5 ($J_{2,3}$ 10 Hz, $J_{3,4}$ 8.5 Hz). The signal for H-4 could not be resolved from other signals, even in the presence of a shift reagent. From these results, the *gulo* configuration is excluded, as smaller values of $J_{2,3}$ and $J_{3,4}$ would then be expected.

In the ^{13}C-n.m.r. spectrum of *R*, two groups of signals were obtained from C-1, C-2, C-3, and C-5, while C-4, C-6, and the carbon atoms of the substituent at O-4 gave single signals (Table II). The chemical shifts of C-1, C-2, C-3, C-5, and C-6 are in good agreement with those obtained from α- and β-D-glucopyranose[11]. The downfield shift (8.6 p.p.m.) of the C-4 signal in *R* relative to that for C-4 in α- and β-D-glucopyranose (122.8 p.p.m. from CS_2) is in agreement with the observed effects on etherification[12]. The positions of the signals from the 1-hydroxy-2-propyl moiety agree with those expected. In order to determine the configurations of the lactic acid residue and the sugar moiety of *A*, the diastereomers of 4-*O*-[2-(1-hydroxy)-propyl]-D-glucose were synthesized.

1,6:3,4-Dianhydro-2-*O*-toluene-*p*-sulfonyl-β-D-galactopyranose[13] (**4**) was treated with racemic 1-allyloxy-2-hydroxypropane under acidic conditions. The

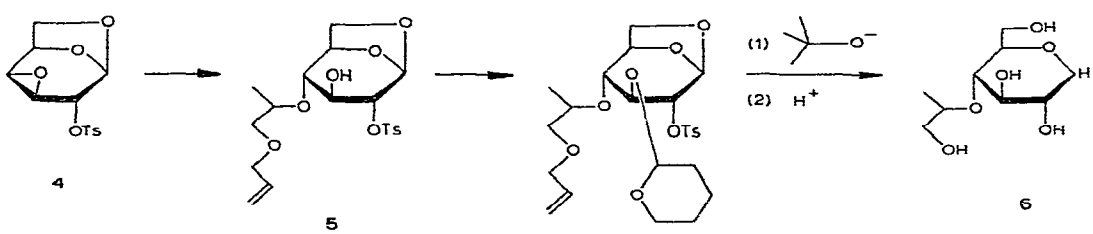
TABLE II

^{13}C-N.M.R. CHEMICAL SHIFTS[a] FOR THE ANOMERIC MIXTURE OF *R*

	R_α	R_β
C-1	100.7	96.8
C-2	121.2	118.6
C-3	119.7	116.6
C-4	114.2	114.2
C-5	122.1	117.4
C-6	132.3	132.3
CH_3	176.1	176.1
H—C—O—	127.4	127.4
CH_2OH	132.4	132.4

[a]Relative to CS_2.

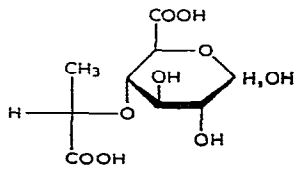
latter substance was prepared from 1,2-dihydroxypropane by partial allylation. The hydroxyl group at C-3 in **5** was protected by acetalation with 1,2-dihydropyran[14]. The allyl group was rearranged to the corresponding prop-1-enyl ether by treatment with potassium *tert*-butoxide[15]. During this treatment, the toluene-*p*-sulfonyl group was simultaneously removed. On subsequent treatment with acid, the blocking groups were removed and the 1,6-anhydro ring was opened, yielding **6**. An analogous route has been used for the introduction of a methyl group at O-4 in D-glucose[16].



The alditol acetate from **6** gave two peaks on g.l.c., the faster of which coincided with the peak given by the alditol acetate of *R*. The m.s. of the alditol acetates were indistinguishable.

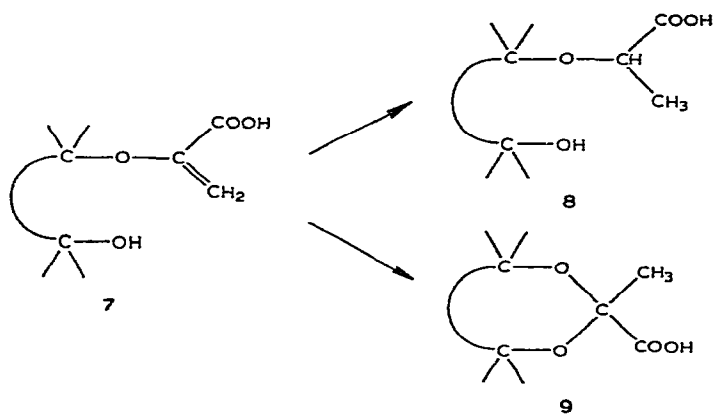
The synthesis was repeated with (*S*)-1-allyloxy-2-hydroxypropane, prepared from L-lactic acid. The amorphous sugar, 4-*O*-[(*S*)-2-(1-hydroxy)propyl]-D-glucose showed $[\alpha]_{578}$ +57°, compared to the value +49° for *R*.

The ^{1}H- and ^{13}C-n.m.r. spectra of the two sugars were superimposable, and the derived alditol acetates were indistinguishable on g.l.c. The combined results therefore demonstrate that the acidic sugar in K 37 is 4-*O*-[(*S*)-1-carboxyethyl]-D-glucuronic acid (*A*).



A

In the biosynthesis of an ether of lactic acid, the first step is most probably the reaction of a hydroxyl group with phosphoenolpyruvate to give an enol ether (**7**). Reduction then yields the ether of lactic acid (**8**). There are only three known natural sugars that are ethers of lactic acid, namely the present ether *A*, muramic acid {2-amino-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-D-glucose}, and the corresponding D-*manno* derivative[17]. The enol ether (**7**) could, however, also react with a neighbouring hydroxyl group with the formation of a cyclic acetal of pyruvic acid (**9**), and such acetals, linked to different sugars and different positions, are common in bacterial polysaccharides.



EXPERIMENTAL

General methods. — G.l.c.–m.s. was conducted using a Varian Mat 311B instrument fitted with a ECNSS-M S.C.O.T. column. The column temperature was 170° and the ionization potential 70 eV. N.m.r. spectra were recorded with a Varian XL 100 spectrometer. Optical rotations were measured at room temperature, using a Perkin–Elmer 141 polarimeter. Evaporations were carried out under reduced pressure, at a bath temperature below 40°.

Methylation analysis[6] and carboxyl reduction[4] of K 37 will be discussed in detail when the full structure of the polysaccharide is reported. Details for the syntheses starting from racemic 1,2-propanediol, which were analogous to those starting from the L isomer, will not be given.

Isolation of 4-O-[(S)-2-(1-hydroxy)propyl]-D-glucose. — Carboxyl-reduced K 37

(500 mg) was hydrolyzed with 0.25M H_2SO_4 at 100° for 18 h. The solution was neutralized with $BaCO_3$ and salts were filtered off. The title compound (55 mg), $[\alpha]_{578}$ +49° (*c* 1.0, water), was isolated by chromatography on Whatman No. 3 filter paper, using ethyl acetate–acetic acid–water (3:1:1), followed by chromatography on Sephadex G 15. The sugar showed R_{Glc} 1.67 (above solvent system) and 1.46 (1-butanol–pyridine–water, 6:3:3), respectively, and M_G 0.3 on electrophoresis in germanate buffer[9].

Acetylation, with acetic anhydride and a trace of sulfuric acid, yielded a main component (α-acetate according to the n.m.r. spectrum) with *T* 3.55, relative to α-D-glucopyranose penta-acetate on an OV-1 column at 150°. M.s. of this product showed, *inter alia*, the following ions: *m/e* 43(100), 45(7), 60(3), 81(5), 97(3), 101(45), 211(1), 215(1), 331(1).

Reduction and acetylation of the sugar yielded on alditol acetate with *T* 3.16, relative to D-glucitol hexa-acetate on an ECNSS-M column at 200°.

The alditol acetate obtained in the methylation analysis of carboxyl-reduced K 37, and derived from the new sugar, showed *T* 1.79, relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, on an SP-1000 glass-capillary column at 215°.

(S)-*1,2-Dihydroxypropane.* — Methyl L-lactate (15 g) in ethyl ether (150 ml) was added to a stirred mixture of $LiAlH_4$ (15 g) in ethyl ether (200 ml), and the mixture was kept at room temperature overnight. Ethyl acetate (50 ml) was added and the mixture neutralized with M H_3PO_4. Salts were filtered off, and washed with methanol, and the combined filtrate and washings were concentrated, and distilled at 10 mmHg. The title compound (8 g) distilled at 70–72° and showed $[\alpha]_{578}$ +15.3° (neat).

(S)-*1-Allyloxy-2-hydroxypropane.* — Silver oxide (4 × 8 g) was added to a mixture of *S*-1,2-dihydroxypropane (10 g), allyl bromide (20 ml), and *p*-dioxane (30 ml), kept at 50° over a period of 4 h. Solids were filtered off and washed with acetone, and the combined filtrate and washings were concentrated at 30° (bath)/ 10 mmHg. The residue was distilled at 31 mmHg, using a spinning-band column. The title compound (3.0 g), $[\alpha]_{578}$ +4.1° (neat), distilled at 68–71° and gave a single peak on g.l.c. (Carbowax 20 M column at 100°).

Part of this product, on oxidation with Jones' reagent, yielded a substance which, according to its ^{1}H-n.m.r. spectrum, was 1-allyloxypropan-2-one.

4-O-[(S)-*2*-(*1-allyloxy*)*propyl*]-*1,6-anhydro-2*-O-*tosyl*-β-D-*glucopyranose* (**5**). — A solution of *S*-1-allyloxy-2-hydroxypropane (3.0 g), conc. H_2SO_4 (0.15 ml), and 1,6-anhydro-2-*O*-tosyl-β-D-glucopyranose (1 g) in benzene (5 ml) was boiled under reflux for 2 h. Chloroform (40 ml) was added, the solution was extracted with water (4 × 10 ml) and concentrated, and the residue was purified by chromatography on a silica gel column (Merck, prepacked, size B), using toluene–ethyl acetate (1:1). The amorphous product (230 mg) showed R_F 0.63 (t.l.c.; silica gel, toluene–ethyl acetate, 1:1) and $[\alpha]_{578}$ −20° (*c* 2.0, chloroform). The n.m.r. spectrum ($CDCl_3$) showed, *inter alia*, peaks at δ 1.15 (d, 3H), 1.70 (s, 1H, disappearing on addition of D_2O), 2.43 (s, 3H), 4.45 (d, 2H), 5.85 (o, 1H), 7.32 (d, 2H), and 7.82 (d, 2H).

4-O-[(S)-2-(*1-hydroxy)propyl*]-D-*glucose* (**6**). — A mixture of **5** (600 mg), dihydropyran (6 ml), and 37% HCl (0.01 ml) was kept at room temperature overnight and then concentrated. The product was dissolved in dry methyl sulphoxide (20 ml), potassium *tert*-butoxide (1.0 g) was added, and the solution was kept at 100° for 30 min. The cooled solution was diluted with water (100 ml), extracted with chloroform (3 × 40 ml), and concentrated. A solution of the product in 0.25M H_2SO_4 (25 ml) was kept at 100° overnight, then neutralized ($BaCO_3$), filtered, and concentrated. The product was further purified by chromatography on paper and Sephadex G 15, as described for the corresponding product obtained from K 37. The pure substance (56 mg) showed $[\alpha]_{578}$ +57° (*c* 1.0, water). The sugar and the derived alditol acetate were indistinguishable from the sugar from carboxyl-reduced K 37 and its alditol acetate on paper chromatography in different solvent systems and g.l.c. on different columns, respectively. The ^1H- and ^{13}C-n.m.r. spectra of the two sugars and of their acetates were superimposable, as were the m.s. of the corresponding acetylated sugars and alditol acetates.

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