Carbohydrate Research, 54 (1977) 253–259

4-*O*-[(*R*)-1-CARBOXYETHYL]-D-GLUCOSE: A NEW ACIDIC SUGAR FROM *Shigella dysenteriae* TYPE 3 LIPOPOLYSACCHARIDE

NIKOLAY K. KOCHETKOV, BORIS A. DMITRIEV, AND VJACHESLAV L. LVOV

N. D. Zelinsky Institute of Organic Chemistry, Academy of Sciences of the U.S.S.R., Moscow (U.S.S.R.)

(Received March 29th, 1976, accepted for publication, August 3rd, 1976)

ABSTRACT

A new acidic monosaccharide, isolated from the *O*-specific lipopolysaccharide of *Sh. dysenteriae* type 3, has been identified as 4-*O*-[(*R*)-1-carboxyethyl]-D-glucose (**1**). The synthesis of **1** involved alkylation of methyl 2,3,6-tri-*O*-benzyl-α(β)-D-glucopyranoside with (*S*)-2-chloropropionic acid, followed by hydrogenolysis and acid hydrolysis. Alkylation with (*R*)-2-chloropropionic acid led to the diastereoisomer of **1**, 4-*O*-[(*S*)-1-carboxyethyl]-D-glucose (**8**).

INTRODUCTION

Recently, we reported on the structural features of the O-specific polysaccharide of *Sh. dysenteriae* type 3 lipopolysaccharide[1], and gave preliminary data[2] on the identification of a new acidic sugar component containing a lactic acid residue.

Three acidic sugars of microbial origin are known which contain monosaccharide and lactic acid moieties, namely muramic acid, the obligatory component of the rigid layer of the microbial cell-wall[3], the *manno* analogue of muramic acid from the peptidoglycan of *Micrococcus lysodeikticus*[4], and 3-*O*-[(*R*)-1-carboxyethyl]-L-rhamnose, the acidic component of *Sh. dysenteriae* type 5 lipopolysaccharide[5].

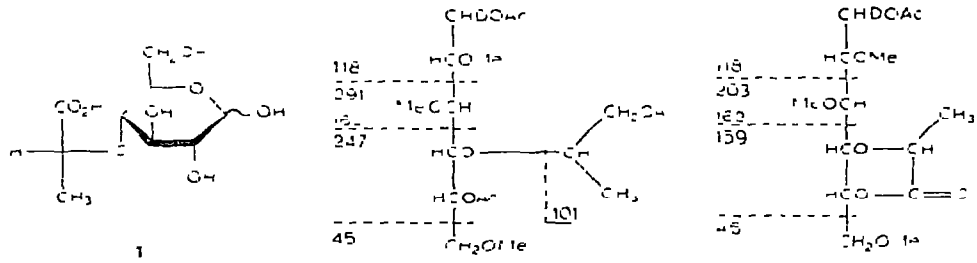
These sugars belong to a new group of natural acidic monosaccharides, and we now describe the identification and synthesis of 4-*O*-[(*R*)-1-carboxyethyl]-D-glucose (**1**), another acidic sugar of this group, isolated from *Sh. dysenteriae* type 3 lipopolysaccharide.

RESULTS AND DISCUSSION

The O-antigenic lipopolysaccharide was isolated from dry cells of *Sh. dysenteriae* type 3 by using hot, aqueous phenol, and then degraded with dilute acetic acid to give the O-specific polysaccharide which was purified by gel-chromatography on Sephadex G-50. Electrophoresis and ion-exchange chromatography data indicated the resulting polysaccharide to be acidic, and the p.m.r. spectrum of its sodium salt

contained signals at δ 2.05 (s, NAc), and 1.47 (d, J 7 Hz, CH*Me*). The polysaccharide was hydrolysed with acid, and the resulting mixture of monosaccharides was fractionated by ion-exchange chromatography to give an acidic product (*Y*) which reacted with spray reagents for reducing sugars. The p.m.r. spectrum of *Y* contained a signal for a CH*Me* group at δ 1.44 (d, J 6 Hz). Since there were no other signals in the high-field part of the spectrum, *Y* was assumed to contain lactic acid attached to a monosaccharide residue by an ether linkage. Treatment of *Y* with boron trichloride, under conditions for the cleavage of ether bonds[6], gave D-glucose which was identified on the basis of D-glucose oxidase and sugar-analyzer data.

The location of the lactic acid residue was determined by methylation analysis. The polysaccharide was methylated by the Hakomori procedure[7], then reduced with lithium aluminium hydride, and hydrolysed. The products were converted into methylated alditol acetates by reduction with sodium borodeuteride followed by acetylation, and then subjected to g.l.c.–m.s. Derivatives of glucose and galactose[1] were identified, together with a compound whose mass spectrum corresponded to the structure **2**; there was an intense peak for the acetoxypropyl group (*m/e* 101) derived from the lactyl residue. This conclusion was confirmed by the shift of the ion at *m/e* 101 to 103 for the corresponding product obtained from the methylated polysaccharide that had been reduced with lithium aluminium deuteride.



1 2 3

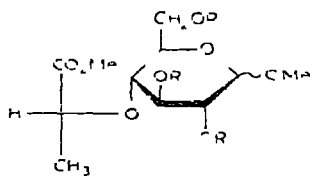
The presence of peaks at *m/e* 45, 102, 118, and 162 indicated that positions 2, 3, and 6 of glucitol were substituted with methoxyl groups, whereas the peak at *m/e* 247 (249 if lithium aluminium deuteride was used) proved that the acetoxypropyl group was attached to position 4. Re-interpretation of the mass-spectral data previously reported[2] is consistent with the lactone structure **3**.

Thus, the acidic component *Y* of *Sh. dysenteriae* type 3 polysaccharide was proved to be 4-*O*-(1-carboxyethyl)-D-glucose. The configuration of the lactic acid residue was established by synthesis.

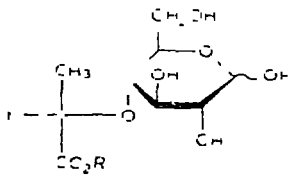
Benzylation of cellobiose in the presence of methylsulphinyl anion[7] afforded the octa-*O*-benzyl derivative, from which cellobiose could be regenerated by hydrogenolysis over palladium–charcoal, thereby indicating the absence of side reactions during benzylation. Methanolysis of the octa-*O*-benzyl derivative yielded a mixture of methyl 2,3,6-tri-*O*-benzyl-$\alpha\beta$-D-glucopyranoside and methyl 2,3,4,6-tetra-*O*-

benzyl-αβ-D-glucopyranoside, which was fractionated by chromatography on silica gel, a small proportion of the pure α anomer of the former compound was obtained.

Methylation of 2,3,6-tri-*O*-benzyl-αβ-D-glucopyranoside followed by debenzylation and acid hydrolysis gave 4-*O*-methyl-D-glucose, which was identified by g l c – m s of its alditol acetate. The tribenzyl ether was alkylated with (*S*)-2-chloropropionic acid (prepared from L-alanine[8]) using *p*-dioxane–sodium hydride under conditions described for the preparation of the *manno* isomer of muramic acid[8]. The crude product was treated with diazomethane to give methyl 2,3,6-tri-*O*-benzyl-4-*O*-[(*R*)-1-(methoxycarbonyl)ethyl]-αβ-D-glucopyranoside (**4**), the anomers of which were isolated by p l c. The structure of each anomer was supported by p m r data. Hydrogenolysis of **4α** and **4β** over palladium-on-charcoal gave methyl 4-*O*-[(*R*)-1-(methoxycarbonyl)ethyl]-α-(**5α**) and -β-D-glucopyranoside (**5β**) respectively. Application in sequence of methylation, acid hydrolysis, borohydride reduction, and acetylation to **8α** and **8β** gave the lactone **3**, and acid hydrolysis of **5α** or **5β** gave **1** which was identical with the naturally occurring compound noted above.



4 α,β-4 R = Bzl
α,β-**5** R = H



7 R = Me
8 R = H

In a parallel series of reactions, methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside was alkylated with (*R*)-2-chloropropionic acid to give methyl 2,3,6-tri-*O*-benzyl-4-*O*-[(*S*)-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside (**6**), and methyl 4-*O*-[(*S*)-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside (**7**), which differed from the diastereoisomer **5α** in chromatographic mobility, $[\alpha]_D$ value, and p m r -spectral data.

Acid hydrolysis of **7** afforded the diastereoisomer of **1**, 4-*O*-[(*S*)-1-carboxyethyl]-D-glucose (**8**), the diastereoisomers **1** and **7** differed in chromatographic properties (ion-exchange and paper chromatography).

Methanolysis of naturally occurring 4-*O*-[(*R*)-1-carboxyethyl]-D-glucose (**1**) gave anomeric methyl glycosides (isolated by p.l c.), the optical rotations of which were closely similar to those of **5α** and **5β** (see Table II). Moreover, a comparison of the p m r data for **5α**, **7**, and the α anomer derived from the naturally occurring sugar further confirmed the (*R*) configuration of the lactic acid moiety in **1** isolated from *Sh. dysenteriae* type 3 polysaccharide.

EXPERIMENTAL

P.c was carried out by the descending method on Futrak FN-11 paper with ethyl acetate–acetic acid–formic acid–water (18:3:1:4). Electrophoresis was performed

on FN-11 paper in 25mM pyridinium acetate buffer (pH 4.5) at 28 V/cm for 90 min. Sugars were detected on paper with alkaline silver nitrate. T.l.c. was performed on Silufol 254 plates (Kavalier, Czechoslovakia), and Silica gel L 100-160 (Czechoslovakia) was used for column chromatography. G.l.c. was performed with a Pye Unicam Series 104 (Model 64) instrument fitted with a dual flame-ionization detector, a glass column (90×0.4 cm) packed with 3% of ECNSS-M on Gaschrom Q (100–120 mesh), and a nitrogen flow-rate of 45 ml/min. G.l.c.–m.s. was performed on a Varian instrument (Gnom MAT 111), by using a column packed with 10% of SE-30 on Chromosorb W (100–120 mesh). Ion-exchange chromatography of sugars was carried out with a column (25×0.5 cm) of DAx4 resin fitted in a SC-2 Technicon sugar analyzer, and elution with 0.5M sodium borate buffer (pH 9 at 55°) at 60 ml/h. Polysaccharide homogeneity was checked by chromatography on a column (10×1 cm) of DEAE-cellulose (Whatman DE-52) and elution with a linear gradient of sodium chloride (0→M) in 0.01M sodium phosphate buffer. The p.m.r. spectrum of the sodium salt of the polysaccharide was recorded in D_2O at 90° with a Varian XL-100 instrument; the spectra of other derivatives were recorded with a Varian DA-60-IL spectrometer.

Optical rotations were measured with a Perkin-Elmer polarimeter, Model 141 at 20°. Melting points were determined with a Kofler apparatus. Solutions were concentrated *in vacuo* at 40°.

The majority of the compounds synthesised were syrups, and the elemental analysis data were not reproducible. The purity of substances was therefore confirmed by chromatographic methods, and identity by p.m.r. spectroscopy.

Isolation of 4-O-[(R)-1-carboxyethyl)-D-glucose (**1**). — The isolation and structural features of *Sh. dysenteriae* type 3 specific polysaccharide have been described[1]. The polysaccharide (300 mg) was hydrolysed with 2M hydrochloric acid (50 ml) for 3 h at 100°. The cooled solution was concentrated, and the residue was dried *in vacuo* over potassium hydroxide and then treated with a 0.5M solution (5 ml) of triethylamine solution in 50% aqueous methanol for 1 h at room temperature. The solution was concentrated and a solution of the residue in water (5 ml) was applied to the top of a column (20×1 cm) of Dowex-1X8 (AcO^-) resin. The column was washed with water (500 ml), and the acid **1** was then eluted with 10% acetic acid (200 ml). The acidic eluate was concentrated to dryness and the traces of acetic acid were removed by coevaporation with water and then with toluene to give **1** (56 mg) as a thick syrup. The chromatographic characteristics of **1** are listed below. The $[\alpha]_D$ value is not a reliable characteristic of **1** because of gradual lactonisation during drying.

Cleavage of acid **1** *with boron trichloride.* — A suspension of **1** (1 mg) in dichloromethane (2 ml) was treated[6] with boron trichloride. The solvents were co-evaporated with methanol (5×5 ml) to leave D-glucose, which was identified by p.c., g.l.c., and ion-exchange chromatography, as well as by oxidation with D-glucose oxidase.

Methanolysis of **1**. — A solution of **1** (40 mg) in M methanolic hydrogen chloride (10 ml) was boiled under reflux for 3 h and then concentrated, and the residue was

dried *in vacuo* over potassium hydroxide. The residue was fractionated by p.l.c. (ether–acetone, 5:2) to give methyl 4-*O*-[(*R*)-1-methoxycarbonyl)ethyl]-α-D-glucopyranoside (**5α**, 25.3 mg) and the corresponding *β* anomer (**5β**, 9 mg). Characteristics of **5α** and **5β** are listed in Table II.

Benzyl 2,3,6-tri-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-αβ-D-glucopyranoside. — A 2M solution of methylsulphinyl anion[7] in methyl sulphoxide (15 ml) was added in portions with stirring to a solution of cellobiose (1 g) in methyl sulphoxide (50 ml, distilled over calcium hydride), and the mixture was stirred for 4 h at room temperature. Benzyl chloride (15 ml) was added dropwise, and the mixture was stirred for another 16 h and then poured into ice–water (100 ml). The product was extracted with chloroform, the extract was washed with water and concentrated, and benzyl chloride was removed at 150°/0.1 Torr. The residue was eluted from silica gel with light petroleum–acetone mixtures, and the semi-crystalline product (2.5 g, 80%) was recrystallized from ethanol to give the title compound, m.p. 81–83°, $[\alpha]_D$ +16° (*c* 1, chloroform).

Anal. Calc. for $C_{68}H_{70}O_{11}$: C, 76.81; H, 6.64. Found: C, 76.80; H, 6.78.

Hydrogenolysis of a small portion of the product over 5% palladium-on-charcoal in acetic acid gave cellobiose identified by p.c. and ion-exchange chromatography.

Methyl 2,3,6-tri-O-benzyl-αβ-D-glucopyranoside. A mixture of chloroform (10 ml), the foregoing benzyl derivatives (2 g), and M methanolic hydrogen chloride (100 ml) was boiled under reflux for 24 h. More (100 ml) methanolic hydrogen chloride was then added and heating was prolonged for another 12 h. The mixture was concentrated and the residue was eluted from silica gel with light petroleum–acetone mixtures, to give methyl 2,3,4,6-tetra-*O*-benzyl-αβ-D-glucopyranoside as a syrupy fraction (870 mg), $[\alpha]_D$ +27.5° (*c* 1, chloroform), and the title compound (510 mg), $[\alpha]_D$ +19.5° (*c* 1.5, chloroform), together with the pure α anomer (200 mg), $[\alpha]_D$ +30° (*c* 1.66, carbon tetrachloride); total yield, 81%. The p.m.r. spectrum of an acetylated sample of the α anomer contained signals at *δ* 1.63 (OAc) and 3.26 (OMe).

Methyl 2,3,6-tri-O-benzyl-4-O-[(R)-1-(methoxycarbonyl)ethyl]-αβ-D-glucopyranoside (**4**). — A solution of the foregoing αβ mixture (480 mg) in *p*-dioxane (10 ml) was stirred with a suspension (500 mg) of sodium hydride in mineral oil for 1 h at 95°. The temperature was then decreased to 65°, (*S*)-2-chloropropionic acid {0.98 g, $[\alpha]_D$ −16.5° (pure liquid, 1-dm tube)} was added, and stirring was continued for 1.5 h. A fresh portion of sodium hydride suspension (1.8 g) was added, and the mixture was stirred for 16 h at 65°, then cooled, cautiously treated with water, and concentrated. A solution of the residue in water was extracted with chloroform and the extract was concentrated. The residue was eluted from silica gel with benzene–ether mixtures. The product (R_F 0.3; t.l.c., benzene–ether, 1:1) was treated in ethereal solution with an excess of diazomethane to give, after fractionation by t.l.c. (benzene–ether, 1:1), **4β** (110 mg) and **4α** (300 mg); total yield, 71%. The characteristics of **4α** and **4β** are given in Tables I and II.

TABLE I

P M R -SPECTRAL DATA

Compound	*Chemical shifts (δ, p p m)*[a]			
	MeCH (*d*, J 7 *Hz*)	*MeO* (*s*)	*AcO* (*s*)	*Ph* (*m*)
4α	1 18	3 29	3 46	7 1
4β	1 18	3 48	3 48	7 1
6	1 20	3 31	3 31	7 1
5α	1 52	3 32	3 60	—
5α (from **1**)	1 52	3 32	3 60	—
7	1 47	3 32	3 55	—

[a]Key s singlet, d doublet, m, multiplet

TABLE II

OPTICAL ROTATION AND T L C DATA

Compound	**4α**[a]	**4β**[a]	**6**[a]	**5α**[a]	**5α** *from* **1**[b]	**6β**[b]	**5β** *from* **1**[c]	**7**[b]
$[\alpha]_D$ (degrees)	+89	+43	+48	+160	+155	+13	+5	+113
R_F	0 53	0 61	0 46	0 36	0 36	0 47	0 43	0 30

[a]In carbon tetrachloride t l c in benzene–ether (1 1) [b]In methanol, t l c in ether–acetone (5 2)

*Methyl 2,3,6-tri-*O*-benzyl-4-*O*-[(S)-1-(methoxycarbonyl)ethyl]-α-*D*-glucopyranoside* (**6**) — This compound (155 mg, 65%) was synthesised by the above method from methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (200 mg) and (*R*)-2-chloropropionic acid {410 mg, $[\alpha]_D$ +17° (pure liquid, 1-dm tube)} The characteristics are given in Tables I and II

*Methyl 4-*O*-[(R)-1-(methoxycarbonyl)ethyl]-α- and -β-*D*-glucopyranoside* (**5**) — A solution of **4α** (250 mg) in methanol (5 ml) was hydrogenolysed over 5% palladium-on-charcoal at room temperature for 16 h, then filtered, and concentrated Purification of the residue by t l c (ether–acetone) gave **5α** (93 mg, 73%) Analogously, **5β** (17 8 mg, 70%) was prepared from **4β** (50 mg) The characteristics of **5α** and **5β** are given in Tables I and II

*Methyl 4-*O*-[(S)-1-(methoxycarbonyl)ethyl]-α-*D*-glucopyranoside* (**7**) — Hydrogenolysis of **6** (120 mg), according to the procedure described above, gave **7** (43 mg, 70%) The characteristics of **7** are given in Tables I and II

*4-*C*-[(R)-1-Carboxyethyl)-*D*-glucose* (**1**) *and its diastereoisomer* **8** — Samples (1 mg) of **5α**, **5β**, and **7** were hydrolysed with 2M hydrochloric acid (1 ml) at 100° for 3 h, the solutions were concentrated, and the residues were dried *in vacuo* over potassium hydroxide to give chromatographically homogeneous samples of **1** (from **5α** and **5β**) and **8** (from **7**), for which the following comparative data were obtained

Compound	R^a_{GalA}	E^b_{Gal}
Natural **1**	1.60	1.47
Synthetic **1**	1.60	1.47
Synthetic **8**	1.03	1.38

[a]Mobility relative to that of D-galacturonic acid in p.c. [b]Elution time relative to that of D-galactose in ion-exchange chromatography.

REFERENCES

1 B. A. DMITRIEV, L. V. BACKINOWSKY, L. V. LVOV, N. K. KOCHETKOV, AND I. L. HOFMAN, *Eur. J. Biochem.*, 50 (1975) 539–547.
2 N. K. KOCHETKOV, B. A. DMITRIEV, L. V. BACKINOWSKY, AND L. V. LVOV, *Bioorg. Chem. U.S.S.R.* 1 (1975) 1238–1240.
3 M. R. J. SALTON, *The Bacterial Cell Wall*, Elsevier, Amsterdam, 1969, p. 133.
4 O. HOSHINO, U. ZEHAVI, P. SINAY, AND R. W. JEANLOZ, *J. Biol. Chem.* 247 (1972) 381–390.
5 N. K. KOCHETKOV, B. A. DMITRIEV, AND L. V. BACKINOWSKY, *Carbohydr. Res.* 51 (1976) 229–237.
6 T. G. BONNER AND E. J. BOURNE, *Methods Carbohydr. Chem.* 2 (1963) 206–207.
7 H. E. CONRAD, *Methods Carbohydr. Chem.*, 6 (1972) 361–364.
8 P. SINAY, M. D. A. HALFORD, M. S. CHOUDHURY, P. H. GROSS, AND R. W. JEANLOZ, *J. Biol. Chem.*, 247 (1972) 391–397.