Carbohydrate Research, 89 (1981) 346–349
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Note

Structure of the dextran synthesised by a new strain of *Leuconostoc mesenteroides*

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(Received October 15th, 1979; accepted for publication, July 24th, 1980)

We have recently isolated and characterised a new strain of *Leuconostoc mesenteroides* from contaminated cane juice which significantly differed from other strains in many of its growth characteristics, *e.g.*, long lag-phase of growth, specific requirement for thiamine and glutamic acid, and kinetic behaviour of dextransucrase[1]. We now describe the structural characterisation of the dextran produced by this strain.

An electrophoretically homogeneous preparation of the dextran (ionic mobility, -0.53×10^{-5} cm^2.s^{-1}.V^{-1}), $[\alpha]_D^{25}$ +203°, on complete hydrolysis, gave D-glucose only. This dextran was methylated by the Haworth[2] and Hakomori[3] procedures, the fully methylated product ($[\alpha]_D^{25}$ +213°) was hydrolysed, and the resulting methylated sugars were converted into peracetylated aldononitriles (PAAN) and characterised by g.l.c.–m.s. (see Table I). The PAAN derivatives of 2,3,4,6-tetra-, 2,3,4-tri-, 2,4-di-, and 3,4-di-*O*-methyl-D-glucose were present in the molar ratios 3.1 : 25.8 : 2.1 : 1. The mass spectra of the PAAN derivatives contained characteristic primary ions at *m/z*

TABLE I

RETENTION TIMES IN G.L.C. OF PERACETYLATED ALDONONITRILES OF METHYLATED D-GLUCOSES OBTAINED BY ACID HYDROLYSIS OF METHYLATED DEXTRAN AND ACIDIC OLIGOSACCHARIDES

Methylated D-glucose	*Column*[a]	*Column*[b]
2,3,4,6-Tetra	1.00	1.00
2,4,6-Tri	1.57	1.32
2,3,4-Tri	2.09	1.69
3,4,6-Tri	1.96	1.51
2,4-Di	3.06	2.57
3,4-Di	3.86	2.04

[a]5% Butanediol succinate. [b]5% Apiezon.

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TABLE II

DATA FOR ACIDIC OLIGOSACCHARIDES OBTAINED FROM CARBOXYL-DEXTRAN

Acidic oligosaccharides	*Yield (mg)*	R_{Glc}[a]	$[\alpha]_D^{25}$ *(water) (degrees)*	*Hydrolysis products of methylated, carboxyl-reduced oligosaccharides*			
				O-Methyl-D-glucose PAAN derivatives (mol)			
				2,3,4,6-tetra	*2,3,4-tri*	*3,4,6-tri*	*2,4,6-tri*
Aldobiouronic acid I	29.2	0.55	+65	1.0		0.97	
Aldobiouronic acid II	13.1	0.46	+57	1.0	0.93		
Aldotriouronic acid	78.6	0.27	+102	1.0	0.96		0.92

[a]In solvent *C*.

205 and 161 (for 2,3,4,6-tetra-*O*-methyl-D-glucose), *m/z* 233 and 189 (for 2,3,4-tri-*O*-methyl-D-glucose), *m/z* 154 (for 2,4-di-*O*-methyl-D-glucose), and *m/z* 142 (for 3,4-di-*O*-methyl-D-glucose).

Isolation of 3 mol. of 2,3,4,6-tetra-*O*-methyl-D-glucose indicated that these molecules constituted end groups. The 26 mol. of 2,3,4-tri-*O*-methyl-D-glucose appeared to have originated from (1→6) linkages. Isolation of 1 mol. of 3,4-di-*O*-methyl-D-glucose and 2 mol. of 2,4-di-*O*-methyl-D-glucose indicated the presence of three branch-points, two from O-3 and one from O-2 in an average repeating-unit of 32 D-glucosyl residues. The presence of α-D linkages in the dextran was revealed by the $[\alpha]_D^{25}$ value of +203°.

To study the nature of branching, the dextran was oxidised[10] and the resulting carboxyl-dextran (89.3% conversion of the non-reducing residues into D-glucuronic acid residues) was partially hydrolysed with acid. The results of methylation analysis of the acidic oligosaccharides so obtained are shown in Table II. The aldobiouronic acids I and II were characterised as 2-*O*-(α-D-glucopyranosyluronic acid)-D-glucose and 6-*O*-(α-D-glucopyranosyluronic acid)-D-glucose, respectively. The triouronic acid, on partial hydrolysis with acid, gave aldobiouronic acid II, nigerose, D-glucuronic acid, and D-glucose. These results suggested that the triouronic acid was *O*-(α-D-glucopyranosyluronic acid)-(1→6)-*O*-α-D-glucopyranosyl-(1→3)-D-glucose. Aldobiouronic acid II might have been derived from the triouronic acid during hydrolysis. As far as we are aware, this is the first report of the isolation, albeit in low yields, of aldobiouronic acid I and the triouronic acid from oxidised dextran.

EXPERIMENTAL

Paper chromatography (p.c.) was performed with *A*, 1-butanol–ethanol–water (4:1:5), *B*, 1-butanol–acetic acid–water (4:1:5); and *C*, ethyl acetate–acetic acid–water (3:1:1); with detection by *p*-anisidine phosphate[4] or by sodium metaperiodate–

benzidine[5]. Peracetylated aldononitriles were prepared as described by Seymour *et al.*[6] and anilides as described by Barker *et al.*[7].

Dextran from L. mesenteroides. — (*a*) *Isolation and purification. L. mesenteroides*, isolated in our laboratory, was grown in Stacey's medium[8] (2 litres) for 72 h at 23°. The fermented medium was diluted two-fold with distilled water, made 35% with respect to ethanol, and centrifuged at 20,000*g* to remove the cells. The ethanol concentration of the supernatant solution was increased to 50%, and the resulting precipitate was collected, dissolved in water, and reprecipitated in the same manner twice. A solution of the precipitate in water was deionised and freeze-dried. The product (30.6 g) had negligible ash (0.112%), a carbohydrate content of 98.88%, and was nitrogen-free. The dextran was subjected to zone electrophoresis on Whatman No. 1 paper with 0.05M borate buffer (pH 9.2) for 6 h at 400 V.

(*b*) *Hydrolysis.* The purified dextran was hydrolysed with 0.05M H_2SO_4 at 100° for 16 h. P.c. (solvents *A* and *B*) of the deionised hydrolysate revealed D-glucose only, which was identified as the phenylosazone. There was 98% conversion of the dextran into D-glucose.

(*c*) *Methylation analysis.* Dextran (6 g) was methylated twice by the Haworth method[2]. A portion (5 g) of this partially methylated dextran (5.4 g; OMe, 30.1%) was methylated by the Hakomori procedure[3]. The product (5.12 g; OMe, 45.6%) showed no significant i.r. absorption between 3200 and 3700 cm^{-1}.

Fully methylated dextran (3.0 g) was hydrolysed with 90% formic acid for 3 h at 100° and then with 0.25M sulphuric acid for 3 h at 100°. The hydrolysate was neutralised with $BaCO_3$, filtered, and concentrated to a syrup (2.729 g). A portion (10 mg) of the hydrolysate was converted[6] into the PAAN derivatives, which were then subjected to g.l.c.–m.s. The molar ratios of methylated PAAN derivatives were determined from the peak areas. A portion of the hydrolysate (800 mg) was fractionated[9] and the following products were obtained.

2,3,4,6-Tetra-*O*-methyl-D-glucose, m.p. 87°, $[\alpha]_D^{25}$ +82.4° (water); lit.[10,11] m.p. 87°, $[\alpha]_D^{22}$ +83.5°. The anilide had m.p. 145°; lit.[7] m.p. 142°.

2,3,4-Tri-*O*-methyl-D-glucose, m.p. 94°, $[\alpha]_D^{25}$ +72.5° (methanol); lit.[11] m.p. 95°, $[\alpha]_D^{22}$ +73.2°. The anilide had m.p. 135°; lit.[7] m.p. 135°.

2,4-Di-*O*-methyl-D-glucose, m.p. 127°, $[\alpha]_D^{25}$ +76.5° (methanol); lit.[7,12] m.p. 125°, $[\alpha]_D^{25}$ +73°. The anilide had m.p. 197°; lit.[7] m.p. 197°.

3,4-Di-*O*-methyl-D-glucose, m.p. 116°, $[\alpha]_D^{24}$ +74° (water); lit.[11,12] m.p. 118°, $[\alpha]_D^{20}$ +74°. The anilide had m.p. 169°; lit.[12] m.p. 168°.

(*d*) *Oxidation.* Dextran (10 g) was oxidised as described by Abbott *et al.*[10]. The glucuronic acid content of the product (7.85 g) was determined according to Whistler *et al.*[13]. The results showed that 89.3% of the non-reducing terminal groups had been converted into glucuronic acid (M_r of the average repeating-unit of the oxidised dextran ~5863). The carboxyl-dextran (5 g) was hydrolysed and the products were fractionated as described by Miyaji *et al.*[9]. The acidic sugar fraction (226 mg) was subjected to p.c. with solvent *C*, to give aldobiouronic acid I (29.2 mg),

aldobiouronic acid II (13.1 mg), and aldotriouronic acid (78.6 mg). A small proportion of glucuronic acid was also detected.

The acidic oligosaccharides (5–10 mg each) were methylated by the Hakomori method[9]. Each methylated product was esterified with diazomethane, reduced with lithium aluminium hydride, and then remethylated by the Purdie method[14]. Completion of methylation was confirmed by i.r. spectroscopy. The products were converted into PAAN derivatives, and characterised by g.l.c.–m.s.

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