895. *Immunopolysaccharides. Part XI.* Structure of an* Acetobacter capsulatum *Dextran.*

By S. A. BARKER, E. J. BOURNE, G. T. BRUCE, and M. STACEY.

A polysaccharide elaborated by *Acetobacter capsulatum* is shown to be a branched dextran, in which the principal glucosidic linkages are α-1 : 6, and the branch points involve positions 1 and 4. The average change length is *ca.* 13 glucose residues.

PREVIOUS workers[1,2] had shown that the polyglucosan synthesised from a starch dextrin by *Acetobacter capsulatum* has serological properties very similar to those of a dextran synthesised from sucrose by a strain of *Leuconostoc mesenteroides*. The high yield of formic acid obtained[2,3] from periodate oxidation of the polysaccharides was indicative of the presence of 1 : 6-linked glucopyranose units. A batch of this polysaccharide, kindly provided by Dr. E. J. Hehre, has now been submitted to further investigation.

The polysaccharide, freed from amylaceous material by dialysis, was not stained by iodine under the standard conditions[4] used for the determination of the blue value of starch components. In agreement with Hehre[2] it was found that the polysaccharide was almost unattacked (*ca.* 1% conversion) by soya-bean β-amylase or salivary α-amylase.

* Part X, *J.*, 1958, 3468.

[1] Hehre and Hamilton, *Proc. Soc. Exp. Biol. Med.*, 1949, **71**, 336.
[2] Hehre, *J. Biol. Chem.*, 1951, **192**, 161.
[3] Hehre, personal communication, 1949.
[4] Bourne, Haworth, Macey, and Peat, *J.*, 1948, 924.

The hydrolysate of the polysaccharide was found to consist almost entirely of glucose (96% conversion), probably with a trace of fructose. The high optical rotation ($[\alpha]_D^{21}$ + 196°) and the infrared absorption spectrum [5] (max. at 837 cm.$^{-1}$ shown by α-anomers in the D-glucopyranose series) both indicated α-glycosidic linkages. Absorption peaks (914 and 766 cm.$^{-1}$) consistent with α-1 : 6-linkages were also present but no absorption at 794 cm.$^{-1}$ attributable to α-1 : 3-linkages was detected. Further evidence for α-1 : 6-linkages was obtained by the isolation of isomaltose, identified as its crystalline β-octa-acetate, from a partial hydrolysate of the polysaccharide. The main trisaccharide component of this partial hydrolysate was probably isomaltotriose since it gave only isomaltose and glucose when treated with N-sulphuric acid for 0·5 hr. at 90°.

Methylations of the dextran with sodium and methyl iodide in liquid ammonia [6] afforded a methyl ether having OMe, 42·7% (a tri-*O*-methylglucosan requires OMe, 45·6%) and ash, 3·1%. A paper chromatogram of a hydrolysate of the trimethyl ether revealed three components, having severally R_F values and staining properties identical with those of 2 : 3 : 4 : 6-tetra-, 2 : 3 : 4-tri-, and 2 : 3-di-*O*-methyl-D-glucose. Quantitative assay on filter paper, essentially as described by Hirst, Hough, and Jones,[7] showed that the percentage molecular proportion of each methyl sugar was: 2 : 3 : 4 : 6-, 7·6; 2 : 3 : 4-, 83·2; and 2 : 3-, 9·2. The *Acetobacter capsulatum* polysaccharide is thus shown to consist essentially of chains of α-1 : 6-D-glucopyranose units (average chain length *ca.* 13 glucose units) which are involved in branching through positions 1 and 4.

It is interesting that no evidence was found for the presence of 1 : 3-branches, which are now known to occur quite widely in dextrans. The low $[\alpha]_D$ value of the dextran in comparison with other dextrans confirms this.

EXPERIMENTAL

Purification of the Dextran.—The dextran (kindly supplied by Dr. E. J. Hehre) had been isolated by fractional precipitation with ethanol and methanol from culture media of *Acetobacter capsulatum* (NCTC 4943) utilising a soluble maize-starch dextrin as its carbon source. The dextran (1% w/w) was freed from traces of amylaceous material by dialysis for 5 days against repeated changes of distilled water and recovered by freeze-drying. After being dried *in vacuo* over phosphoric oxide at 60°, the dextran had ash, 0·2%; N, 0·3%; $[\alpha]_D^{21}$ +196° (*c* 0·18 in N-sodium hydroxide). Paper chromatography of the purified dextran, with butanol–ethanol–water–ammonia (40 : 10 : 49 : 1) as solvent and aniline hydrogen phthalate,[8] naphtharesorcinol,[9] and ammoniacal silver nitrate [10] as sprays failed to reveal any oligosaccharides. The dextran was not stained by iodine under standard [4] blue-value conditions.

Acidic Hydrolysis of the Purified Dextran.—The dextran (20 mg.) was hydrolysed with 1·4N-sulphuric acid (1 c.c.) at 100° for 5 hr. After removal of ions,[11] the neutral hydrolysate was examined by paper chromatography as above; the main component had an R_F value equal to that of glucose; traces of compounds which were probably fructose and hydroxymethylfurfuraldehyde were also detected. Determination [12] of the glucose produced when another sample of the dextran was hydrolysed for 5 hr. with 1·5N-sulphuric acid at 100° showed that the extent of the conversion was 96%.

Partial Acidic Hydrolysis of the Dextran.—The dextran (0·838 g.) was partially hydrolysed with N-sulphuric acid (37 c.c.) at 85° for 11 hr., $[\alpha]_D^{20}$ having then fallen to +131°. The acidic solution of saccharides was fractionated on a charcoal column [13] which was washed as shown in Table 1. Each eluate was neutralised with barium carbonate (when necessary), filtered, and concentrated to a syrup, and the saccharides were extracted with hot methanol or

[5] Barker, Bourne, Stacey, and Whiffen, *J.*, 1954, 171.
[6] Freudenberg, Boppel, and Meyer-Delius, *Naturwiss.*, 1938, **26**, 123.
[7] Hirst, Hough, and Jones, *J.*, 1949, 928.
[8] Partridge, *Nature*, 1949, **164**, 443.
[9] *Idem, Biochem. J.*, 1948, **42**, 238.
[10] *Idem, Nature*, 1946, **158**, 270.
[11] Consden, Gordon, and Martin, *Biochem. J.*, 1947, **41**, 590.
[12] Bourne, Donnison, Haworth, and Peat, *J.*, 1948, 1687.
[13] Whistler and Durso, *J. Amer. Chem. Soc.*, 1950, **72**, 677.

hot 50% aqueous methanol. Thereafter, they were obtained as freeze-dried powders and analysed by paper chromatography.

TABLE. 1. *Partial acidic hydrolysis of the dextran.*

Eluant	Fraction	Wt. (g.)	Probable sugar components
Water	A	0·252	Glucose, fructose (*ca.* 5%)
5% Ethanol (1)	B	0·129	Isomaltose
5% Ethanol (2)	C	0·102	Trisaccharide
10% Ethanol (1)	D	0·015	Trisaccharide, tetrasaccharide
10% Ethanol (2)	E	0·069	Tetrasaccharide
10% Ethanol (3)	F	0·030	Tetrasaccharide, pentasaccharide
10% Ethanol (4)	G	0·039	Pentasaccharide
35% Ethanol	H	0·176	Higher saccharides

Part (0·23 g.) of fraction A was characterised by conversion into penta-*O*-acetyl-β-D-glucopyranose (0·10 g.), m. p. and mixed m. p. 128—130°. Fraction B had $[\alpha]_D^{19}$ (uncorrected for ash) +111·1° (equil.) (*c* 0·41 in H_2O) (ash, 3·2%); Montgomery, Weakley, and Hilbert [14] report $[\alpha]_D^{25}$ +120° (equil.) (*c* 1·2 in H_2O), for isomaltose. A portion (0·048 g.) of fraction B, treated with acetic anhydride (0·5 c.c.) and fused sodium acetate (0·013 g.), gave β-isomaltose octa-acetate (0·036 g.), m. p. and mixed m. p. 144—146° (Found: C, 49·7; H, 5·8. Calc. for $C_{28}H_{38}O_{19}$: C, 49·6; H, 5·6%). Fraction C, after partial hydrolysis with N-sulphuric acid at 90° for 0·5, 1·0, and 2·0 hr., gave isomaltose and glucose (identified by paper chromatography).

Methylation of the Purified Dextran.—The dextran (1·1 g.) was treated with sodium and methyl iodide in liquid ammonia at −70° to −80° by a procedure similar to that of Freudenberg, Boppel, and Meyer-Delius.[6] After four additions of the methylating reagents (reaction times; sodium, 4—6 hr.; methyl iodide, 0·5 hr.) had been made, the ammonia was allowed to evaporate, with exclusion of moisture, the last traces being removed at 75°/20 mm. After dialysis the product (1·2 g.) was freeze-dried. This procedure was repeated thrice more, affording methyl ethers having OMe, 41·7%, 41·2%, and 38·8%, respectively. The final product was purified by Soxhlet-extraction with light petroleum (b. p. 60—80°) to remove grease and was then extracted with chloroform and dried. The methylated dextran (0·71 g.) showed OMe, 42·7% and ash, 3·1% (Calc. for $C_9H_{16}O_5$: OMe, 45·6%).

Identification of the Methyl Sugars obtained from the Methylated Dextran.—The polysaccharide ether (0·055 g.) in chloroform (1 c.c.) was treated with 8% (w/w) methanolic hydrogen chloride (1 c.c.) at 100° for 8 hr. and then, after removal of the solvent, with 4% hydrochloric acid (5·5 c.c.) at 100° for 6 hr. Paper-chromatographic analysis of the resulting methyl sugars revealed three components having R_F values and staining properties [8] identical with those of 2 : 3-di-*O*-methylglucose (R_G 0·69, brown stain), 2 : 3 : 4-tri-*O*-methylglucose (red stain), and 2 : 3 : 4 : 6-tetra-*O*-methylglucose and distinguishable from 2 : 4-di-*O*-methylglucose (R_G 0·64, red stain), 3 : 4-di-*O*-methylglucose (R_G 0·65, brown stain), and 2 : 3 : 6-tri-*O*-methylglucose (brown stain).

Determination of the Methyl Sugars obtained from the Methylated Dextran.—The remainder of the hydrolysate mentioned above was analysed by a method which was essentially that used by Hirst, Hough, and Jones [7] with minor modifications introduced by Barker, Bourne, and Wilkinson.[15] The results of three independent quantitative determinations based on oxidation with hypoiodite, are given in Table 2.

TABLE 2. *Quantitative analysis of the methyl sugars.*

Glucose derivative	Molecular composition (%) indicated by assay no. I	II	III	Mean
2 : 3-Di-*O*-methyl	9·3	9·1	9·3	9·2
2 : 3 : 4-Tri-*O*-methyl	83·4	83·3	83·0	83·2
2 : 3 : 4 : 6-Tetra-*O*-methyl	7·4	7·6	7·7	7·6

We thank Dr. E. J. Hehre for the *Acetobacter capsulatum* polysaccharide. One of us (G. T. B.) thanks Courtaulds' Scientific and Educational Trust Fund for financial assistance.

CHEMISTRY DEPARTMENT, THE UNIVERSITY, BIRMINGHAM, 15.
CHEMISTRY DEPARTMENT, ROYAL HOLLOWAY COLLEGE,
ENGLEFIELD GREEN, SURREY. [*Received, July 24th,* 1958.]

[14] Montgomery, Weakley, and Hilbert, *J. Amer. Chem. Soc.*, 1949, **71**, 1682.
[15] Barker, Bourne, and Wilkinson, *J.*, 1950, 3027.