Immunopolysaccharides. Part V. Structure of a Modified* Betacoccus arabinosaceous *Dextran.*

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A polyglucosan, elaborated when *Betacoccus arabinosaceous* (Birmingham strain) is grown in a magnesium-deficient medium, is shown to be a dextran, in which the principal glucosidic linkages are α-1 : 6, a small proportion of the glucose residues being involved in branching of the 1 : 3-type. The average chain-length is 40—50 units.

It has been proved (Barker, Bourne, Bruce, and Stacey, *Chem. and Ind.*, 1952, 1156; Barker, Bourne, Bruce, Neely, and Stacey, *J.*, 1954, 2395) that *Betacoccus arabinosaceous* (Birmingham strain) normally produces a highly branched dextran (average chain-length, 6—7 glucose residues), in which the branch points involve positions 1 and 3. However, in the autumn of 1951, one of us (A. E. J.) isolated samples of dextran in an unusual granular form during commercial production of the polysaccharide, using the same organism. It is one of these samples which is the subject of the present communication.

The organism, which appeared to have the usual culture characteristics (Stacey and Swift, *J.*, 1948, 1555), was grown on an 800-gallon scale in a yeast–sucrose–phosphate stock medium. Formation of the granular dextran introduced difficulties in the operation of the plant, which made a detailed structural study desirable. In addition, the phenomenon was of considerable biochemical interest because it was later established that it arose through magnesium deficiency, caused by removal of a magnesium-rich precipitate during the preparation of the sterile medium. The ability of the organism to produce the usual

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highly branched dextran was regained when it was subsequently cultured on magnesium-rich media.

The dextran, which was virtually free from lower saccharides, minerals, and nitrogenous materials, had $[\alpha]_D^{19}$ +194° in N-sodium hydroxide, compared with +202° for the normal highly branched dextran (Barker *et al.*, *loc. cit.*, 1954). These values are consistent with Jeanes and Wilham's observation (*J. Amer. Chem. Soc.*, 1952, **74**, 5339) that dextrans with smaller proportions of 1 : 3-linkages have lower rotations. Paper-chromatographic analysis of a hydrolysate showed only glucose, which was later crystallized; the extent of the conversion was shown by cuprimetric titration to be 97%.

The dextran was methylated with sodium hydroxide and methyl sulphate, by a procedure similar to that employed by Peat, Schlüchterer, and Stacey (*J.*, 1939, 581). The product had OMe, 45·2% (a tri-*O*-methylglucosan requires OMe, 45·6%). A paper chromatogram of a hydrolysate of the trimethyl ether revealed three components, having R_F values and staining properties identical with those of 2 : 3 : 4 : 6-tetra-*O*-methyl-, 2 : 3 : 4-tri-*O*-methyl-, and 2 : 4-di-*O*-methyl-glucose, respectively.

The identity of the methylglucoses was confirmed by fractionation of a hydrolysate of the methylated dextran on a silica gel column (Bell, *J.*, 1944, 473). The 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose was characterized as the crystalline α-anomer and as the aniline derivative, and the tri-*O*-methyl-D-glucose by conversion into 2 : 3 : 4-tri-*O*-methyl-*N*-phenyl-D-glucosylamine. The 2 : 4-di-*O*-methyl-D-glucose was present in insufficient amount for crystallization and was characterized by comparing it, chromatographically and ionophoretically, with an authentic sample (supplied by Dr. D. J. Bell) and with specimens of 2 : 3- and 3 : 4-di-*O*-methyl-D-glucose.

The mixture of saccharides produced by partial hydrolysis of the dextran was fractionated on a charcoal column (Whistler and Durso, *J. Amer. Chem. Soc.*, 1950, **72**, 677). *iso*Maltose, identified as its crystalline β-octa-acetate, constituted the major portion of the disaccharide fraction, only a trace of a second disaccharide, having the R_F value of nigerose, being detected. The *iso*maltotriose fraction was not accompanied by any other detectable trisaccharide.

The dextran consumed 1·96 mol. of periodate and produced 0·94 mol. of formic acid per anhydroglucose unit, when oxidized for 112 hr. at 26°. On the assumption that only 1 : 3-branches were present, the former figure indicated a chain-length of approximately 50 glucose residues, while the latter corresponded to a chain-length of only *ca.* 17. This low value for formic acid (0·94 mol. instead of 0·98 mol.) may well be attributable to the inaccuracy of the determination in the presence of oxidized polysaccharide. The chain-length of 50 is in reasonable agreement with the value (40) calculated from the proportion of "tetra" in the hydrolysate of the methylated dextran. Again, hydrolysis of the periodate-oxidised dextran gave, *inter alia*, a component with an R_F value equal to that of glucose, in an amount estimated visually, by comparison with standards, to correspond to a chain-length of *ca.* 50 units. The appearance of this sugar confirmed the presence of 1 : 3-branch points.

Finally, the infrared absorption spectrum of the dextran was consistent with its negligible degree of branching (cf. Barker, Bourne, Stacey, and Whiffen, *J.*, 1954, 171). There were absorption peaks at 917 and 768 cm.$^{-1}$ (α-1 : 6-linkages) and at 841 cm.$^{-1}$ (shown by α-anomers in the D-glucopyranose series), no absorption at *ca.* 794 cm.$^{-1}$ (α-1 : 3-linkages) being detectable. The normal highly branched dextran produced by *Betacoccus arabinosaceous* does give an absorption band at 794 cm.$^{-1}$ (Barker *et al.*, *loc. cit.*, 1954).

Thus it is clear that the degree of branching in the dextran synthesized by this organism is dependent on the composition of the culture medium. Magnesium favours the production of branches, possibly because it is a component of the enzyme system responsible for their formation.

Experimental

Isolation of the Dextran.—*Betacoccus arabinosaceous* (Birmingham strain) was grown on a yeast–sucrose–phosphate stock medium in 800-gallon tanks for 2 days. The viscous mass was degraded at pH 7·0 and 160°, in the presence of sodium sulphite and calcium carbonate (cf.

Stacey and Pautard, *Chem. and Ind.*, 1952, 1058), until the mean molecular weight of the dextran, as determined by viscosity measurements, was 200,000. The bacterial debris was adsorbed on a calcium phosphate flock and filtered off. The dextran was precipitated several times with acetone and dried at 60° *in vacuo* over phosphoric oxide; it had ash, 0·07; N, 0·4%; $[\alpha]_D^{19}$ +194° (*c*, 1·0 in N-NaOH).

Paper chromatography of the dextran, with the upper phase of *n*-butanol–ethanol–water–ammonia (40 : 10 : 49 : 1) as the solvent, and the usual naphtharesorcinol, aniline hydrogen phthalate, and ammoniacal silver nitrate sprays, failed to reveal any oligosaccharides.

Acidic Hydrolysis of the Dextran.—The polysaccharide (20 mg.) was hydrolysed with 1·4N-sulphuric acid (2 c.c.) at 100° for 5 hr. in a sealed tube. After the removal of ions by Consden, Gordon, and Martin's method (*Biochem. J.*, 1947, **41**, 590), the neutral hydrolysate was examined by paper chromatography, as described above; the single component had an R_F value equal to that of glucose.

Another sample of the polysaccharide was hydrolysed for 5 hr. with 1·4N-sulphuric acid at 100°, and the glucose produced was determined by cuprimetric titration (Bourne, Donnison, Haworth, and Peat, *J.*, 1948, 1687). After a small correction (2%) for the loss in reducing power which occurs when glucose itself is treated with acid under these conditions (Pirt and Whelan, *J. Sci. Food Agric.*, 1951, **2**, 224), the extent of the conversion corresponded to 97%.

After hydrolysis of another sample (1·00 g.) of the dextran, during which $[\alpha]_D$ fell to +52° (constant), α-D-glucose was obtained, having m. p. and mixed m. p. 142—144°. This was further characterized as penta-*O*-acetyl-β-D-glucopyranose, m. p. and mixed m. p. 131—133°.

Methylation of the Dextran.—The dextran (8·0 g.) was dissolved in 30% sodium hydroxide solution (100 c.c.) and treated at 40—50° with methyl sulphate (180 c.c.) and 30% sodium hydroxide solution (250 c.c.), following the procedure of Peat, Schlüchterer, and Stacey (*loc. cit.*). After fifteen such methylations the crude methyl ether (5·2 g.) was purified by extraction with chloroform, concentration to a syrup, and precipitation of the methylated dextran with an excess of light petroleum. The product had ash, 0·12; OMe, 45·2% (Calc. for $C_9H_{16}O_5$: OMe, 45·6%).

Identification of the Methyl-sugars obtained from the Methylated Dextran.—The polysaccharide ether (0·152 g.), in dry chloroform (5 c.c.), was treated with 10% methanolic hydrogen chloride (5 c.c.) in a sealed tube at 100° for 6 hr., and then, after removal of the solvent, with 4% hydrochloric acid (25 c.c.) at 100° for 6 hr., the optical rotation then being constant. The resulting solution of methylglucoses was freed from ions and concentrated to a syrup. A paper chromatogram of the hydrolysate, irrigated for 24 hr. as previously described, showed three components when sprayed with aniline hydrogen phthalate (Partridge, *Nature*, 1949, **164**, 443). The components had R_F values and staining properties identical with those of 2 : 4-di-, 2 : 3 : 4-tri-, and 2 : 3 : 4 : 6-tetra-*O*-methylglucose. The di- and tetra-methyl ethers were present in only small, apparently equal, amounts.

Isolation of the Methyl-sugars obtained from the Methylated Dextran.—The methylated dextran (2·000 g.), after methanolysis and hydrolysis, gave a syrup which was freed from ions and then fractionated by Bell's method (*loc. cit.*) on a column of silica gel (Gordon, Martin, and Synge, *Biochem. J.*, 1943, **37**, 79). Three fractions (I, 0·050 g.; II, 1·801 g.; III, 0·048 g.) were obtained; paper chromatography and ionophoresis showed them to contain, respectively, 2 : 3 : 4 : 6-tetra-, 2 : 3 : 4-tri-, and 2 : 4-di-*O*-methyl-glucose.

Fraction I, crystallized from ether, gave 2 : 3 : 4 : 6-tetra-*O*-methyl-α-D-glucose (0·020 g.), m. p. and mixed m. p. 85—88°. Its infrared spectrum over the range 1400—700 $cm.^{-1}$ was identical with that of an authentic specimen. The syrup obtained by concentration of the mother-liquors, treated with aniline (0·30 c.c.) in ethanol (10 c.c.) under reflux for 2 hr., gave 2 : 3 : 4 : 6-tetra-*O*-methyl-*N*-phenyl-D-glucosylamine (0·010 g.), m. p. and mixed m. p. 133—135°. Peat, Schlüchterer, and Stacey (*loc. cit.*) reported m. p. 135—136°.

Fraction II (0·100 g.), refluxed with aniline (0·60 c.c.) in ethanol (1·00 c.c.) for 4 hr., gave crystals, which were recrystallized from ether–light petroleum (b. p. 40—60°). This material (0·041 g.) had m. p. 147—149°, not depressed on admixture with an authentic specimen of 2 : 3 : 4-tri-*O*-methyl-*N*-phenyl-D-glucosylamine. Peat, Schlüchterer, and Stacey (*loc. cit.*) gave m. p. 145—146°.

Fraction III had R_F and M_G values identical with those of authentic 2 : 4-di-*O*-methylglucose; it could be distinguished on the same basis from 2 : 3- and 3 : 4-di-*O*-methylglucose (cf. Barker *et al.*, *loc. cit.*, 1954).

Partial Hydrolysis of the Dextran.—The dextran (2·73 g.) was partially hydrolysed with N-sulphuric acid (100 c.c.) at 100° for 1·75 hr., $[\alpha]_D^{17}$ having then fallen to 114°. The solution was

neutralized with barium carbonate, filtered, and then fractionated on a charcoal column (Whistler and Durso, *loc. cit.*), which was washed as shown in the Table. The eluates were filtered, concentrated, freeze-dried, and then analysed by paper chromatography.

Partial acidic hydrolysis of the dextran.

Eluant	Fraction	Wt. (g.) of product	Probable sugar components (by paper chromatography)
Water, 1600 c.c.	A	0·789	Glucose
5% Ethanol (1), 1500 c.c.	B	0·256	*iso*Maltose
5% Ethanol (2), 1000 c.c.	C	trace	Nigerose
10% Ethanol, 1600 c.c.	D	0·150	Trisaccharide
15% Ethanol, 1800 c.c. ..... 35% Ethanol, 2000 c.c.	E	1·483	Higher saccharides

Fraction A was further characterized by crystallization to give α-D-glucose, m. p. and mixed m. p. 142—144°, $[\alpha]_D^{18}$ +110° ⟶ +52° (*c*, 1·02 in H_2O), and by conversion into penta-*O*-acetyl-β-D-glucopyranose, m. p. and mixed m. p. 130—132°. Fraction B had $[\alpha]_D^{20}$ + 121·8° (corrected for ash) (*c*, 0·14 in H_2O), ash 5·04%; Montgomery, Weakley, and Hilbert (*J. Amer. Chem. Soc.*, 1949, **71**, 1682) reported $[\alpha]_D^{25}$ +120° (equil.) for *iso*maltose. A portion of this product, treated with acetic anhydride and fused sodium acetate at 100° for 1 hr., gave β-*iso*maltose octa-acetate, m. p. and mixed m. p. 140—143°. Fraction D had the same R_F value as *iso*maltotriose, and, after partial hydrolysis with N-sulphuric acid for 0·5 hr. at 100°, *iso*maltotriose, *iso*maltose, and glucose were identified by paper chromatography. This trisaccharide had $[\alpha]_D^{19}$ +145° (*c*, 0·24 in H_2O), ash negligible. Jeanes, Wilham, Jones, Tsuchiya, and Rist (*J. Amer. Chem. Soc.*, 1953, **75**, 5911) reported $[\alpha]_D$ +145° for *iso*maltotriose.

Periodate Oxidation of the Dextran.—The procedure adopted was essentially that of Jeanes and Wilham (*J. Amer. Chem. Soc.*, 1950, **72**, 2655), which incorporates Fleury and Lange's method (*J. Pharm. Chim.*, 1933, **17**, 107, 196) for the determination of the quantity of periodate consumed, and that of Halsall, Hirst, and Jones (*J.*, 1947, 1427), with certain modifications, for the determination of the quantity of formic acid produced. The number of moles of sodium periodate consumed per mole of anhydroglucose was: 16·5 hr., 1·63; 22·5 hr., 1·72; 41 hr., 1·93; 112 hr., 1·96; and the corresponding figures for moles of formic acid produced were 16·5 hr., 0·76; 41 hr., 0·91; 112 hr., 0·94.

Ethylene glycol was added to destroy the excess of periodate, and sodium hydroxide to neutralize the formic acid in a solution of dextran (0·1882 g.) which had been submitted to periodate oxidation for 112 hr. under the conditions given above. The oxidized polysaccharide was obtained after dialysis and concentration under reduced pressure. After hydrolysis with 1·4N-sulphuric acid (5 c.c.) at 100° for 9 hr., the solution was neutralized with barium carbonate, filtered, and concentrated. The syrup (0·104 g.) obtained was dissolved in water (2 c.c.) and chromatographed. It showed a component having an R_F value equal to that of glucose. The amount of glucose was determined approximately by visually comparing the intensity of the spot with known standards. The amount of glucose indicated that one glucose unit in approximately every fifty of the dextran was linked through position 3.

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