132. *Polysaccharides. Part XXX. The Polysaccharide produced from Sucrose by* Betabacterium Vermiformé (*Ward–Mayer*).

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The dextran synthesised from sucrose by *Betabacterium vermiformé* (Ward–Mayer) is shown to be constituted of α-glucopyranose residues united by 1 : 6-glucosidic links. This dextran is different from the *Leuconostoc dextranicum* product (see previous paper) in that the basal chains consist of only 25 glucose units. These chains are united to form larger molecular aggregates in possibly the same way as the basal chain units of starch are combined.

Betabacterium vermiformé (Ward–Mayer), an organism recently isolated from the "Tibi-complex", has been shown by H. D. Mayer (private communication from Prof. A. J. Kluyver) and by Daker and Stacey (*Biochem. J.*, **1938**, **32**, 11) to produce a polysaccharide when grown on a substrate containing sucrose. The polysaccharide closely resembles in its properties the "dextrans" produced by the *Leuconostoc* species and the present investigation shows it to be constituted on the same general plan. Freshly isolated "*vermiformé*" dextran contains a small proportion of a nitrogenous constituent (N, 0·2%) and is insoluble in water. It becomes soluble in water, $[\alpha]_D$ + 180°, after treatment with either acid or alkali and simultaneously the nitrogenous constituent is removed (Daker and Stacey, *loc. cit.*).

Methylation of the dextran was effected by methods similar to those described for the *Leuconostoc* dextran. An essentially homogeneous methylated product was thus prepared (OMe, 44·0%) and in this instance it was possible, by carrying out the hydrolysis with 50% aqueous acetic acid containing only 2% of concentrated hydrochloric acid, to avoid the undesirable production of lævulic acid.

The conversion of the hydrolysis products into the corresponding methylglucosides and the fractional distillation of the latter were carried out by standard methods. The main hydrolysis product (90% yield) was thus shown to be 2 : 3 : 4-trimethyl glucopyranose, which crystallised in the form of its β-methylglucoside. In addition, 2 : 3 : 4 : 6-tetramethyl glucopyranose was isolated as the "end-group" in about 5% yield. The amount of dimethyl glucose present was not significant.

These findings show that "*vermiformé*" dextran is a terminated chain molecule of about 25 glucose units. The union between mutually linked units is 1 : 6-glucosidic and the

change in optical rotation on hydrolysis ($[\alpha]_D$ 214° ⟶ + 69°) indicates that these linkages are mainly of the α-type.

The particle weight of the methylated "*vermiformé*" dextran has been measured by osmotic pressure methods by Mr. W. T. Chambers in this laboratory and corresponds to a chain length of about 500 glucose units. In this respect there is a striking similarity between the "*vermiformé*" dextran and starch. With each polysaccharide, it is found that the molecular weight determined by physical means is a large multiple of that shown by the end-group method.

Experimental.

Soluble "*vermiformé*" dextran was prepared and purified by the methods described by Daker and Stacey (*loc. cit.*). It is a white granular powder, $[\alpha]_D$ + 180° in water, ash 0·23%. Hydrolysis with N-sulphuric acid gives crystalline *d*-glucose in 93% yield. The iodine number, 4·7, corresponds to a chain of about 27 glucose units.

Methylation.—The dextran was methylated in 10 g. portions with 30% sodium hydroxide solution and methyl sulphate in the presence of acetone at 40°. After four treatments the partly methylated dextran (OMe, 37·0%) separated from the methylation mixture as a granular powder. This product was allowed to swell in dioxan and methylated at 40° with methyl sulphate and potassium hydroxide solution (40%). Six methylations with these reagents gave a product (OMe, 42·0%), which was insoluble in water or alkali and extremely resistant to further methylation. Consequently it was found necessary between each successive treatment to isolate the product in a finely divided state. This was best effected by precipitation from dilute chloroform solution with light petroleum. A product was thus obtained having OMe 44·0%, $[\alpha]_D^{20°}$ + 210° in chloroform (*c*, 1·1), and ash 0·5%. The methylated dextran (50 g.) was separated from chloroform solution by the addition of light petroleum into the following fractions, the properties of which served to demonstrate its homogeneity:

Fraction.	Yield, g.	$[\alpha]_D^{20°}$ in $CHCl_3$.	% OMe.	% Ash.	$\eta_{sp.}$ in *m*-cresol.
I	10	+210°	43·8	2·0	0·16
II	20	+214	44·0	0·31	0·13
III	19	+220	44·0	0·20	0·18

Hydrolysis.—The methylated dextran (45 g.) was dissolved in 50% acetic acid (1600 c.c.) containing 2·5% of concentrated hydrochloric acid and heated on a boiling water-bath. Hydrolysis was complete in 6 hours ($[\alpha]_D$ + 214° ⟶ + 69°). The solution was neutralised with barium carbonate, concentrated in a vacuum to half volume, and extracted with chloroform (8 times). The chloroform extract was dried over anhydrous magnesium sulphate and filtered. After distillation of the chloroform a syrup (A) (20 g.) remained. The aqueous solution was evaporated to dryness, leaving a solid residue from which a syrup (B) (23·3 g.) was isolated by further chloroform extraction. Syrups A and B were separately converted into the methylglucosides by boiling with methyl-alcoholic hydrogen chloride (2%). The glucosides were fractionally distilled in a high vacuum from a Widmer flask. Repeated distillation enabled the following fractions to be obtained:

Fraction.		Yield, g.	Bath temp. at 0·01 mm.	$n_D^{20°}$.	% OMe.	Properties.
A	1a	3·00	95°	1·4470	57·0	Mobile syrup
	2a	13·30	125—130	1·4560	51·6	Crystalline
	3a	0·61	140—160	1·4662	42·5	Viscous syrup
	4a	3·0	Residue	—	—	Hard glass
B	1b	13·40	125	1·4560	51·1	Crystalline
	2b	8·24	128—130	1·4560	51·3	,,
	3b	0·49	130—160	1·4690	42·7	Viscous syrup

The undistilled residue (4a) was rehydrolysed and redistilled:

R_1	1·12	130	1·4564	52·4	Crystalline
R_2	0·90	130—140	1·4590	50·1	,,

Fraction 1a was redistilled several times and it appeared to consist of a constant-boiling mixture. It was hydrolysed with hydrochloric acid (5%); the hydrolysate, which partly crystallised, was drained on a porous tile, recrystallised from ether–light petroleum, and identified as 2 : 3 : 4 : 6-tetramethyl glucopyranose, m. p. 84—85° alone or in admixture with an authentic specimen. The tile was extracted with boiling chloroform; this was distilled off, leaving a syrup, which was converted into the methylglucosides and distilled in a high vacuum. No

difficulty was now experienced in isolating the remainder of the tetramethyl methylglucoside, OMe 60·0%, $n_D^{20°}$ 1·4433. A total of 1·95 g. of tetramethyl glucose was isolated; this amount corresponds to a terminated chain of about 25 glucose units in agreement with the value indicated by the iodine number.

The crystalline material from fractions 2a, 1b, 2b, R_1, and R_2 was combined and recrystallised from ether–light petroleum. It showed OMe 51·8%, $[\alpha]_D^{21°}$ − 20° in water (*c*, 1·3), m. p. 93—94° alone or in admixture with 2 : 3 : 4-trimethyl β-methylglucopyranoside.

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