## 131. *Polysaccharides. Part XXIX. Constitution of the Dextran produced from Sucrose by* Leuconostoc Dextranicum (Betacoccus Arabinosaceous Haemolyticus).

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The constitution of the dextran synthesised by *Leuconostoc dextranicum* has been established by examination of the products of hydrolysis of the methylated polysaccharide. It is a polyglucose in which α-glucopyranose units are linked in the 1 : 6-positions to form a terminated chain. An " end-group," isolated as tetramethyl glucose anilide, indicates a chain length of not more than 550 glucose units and osmotic pressure measurements indicate that the length of the chain is not less than 200 glucose units.

Metabolic activity and polysaccharide formation by organisms of the *Leuconostoc* species (the causative agents of " slime " in the sugar industry) have been extensively studied by numerous authors (see, for example, Tarr and Hibbert, *Canadian J. Res.*, 1931, **5**, 414; Carruthers and Cooper, *Biochem. J.*, 1936, **30**, 1001). These polysaccharides, which can readily be obtained in *ca.* 20% yields from sucrose, are dextrorotatory polymers of *d*-glucose and have been termed " dextrans." In the present communication an account is given of the chemical investigation of the structure of the water-soluble dextran synthesised by *Leuconostoc dextranicum* (Stacey and Youd, *Biochem. J.*, 1938, **32**, 1943).

Methylation of the polysaccharide was carried out with methyl sulphate and sodium hydroxide, with the addition, in the later stages, of dioxan, which caused the partly methylated dextran to swell and thereby assisted the methylation. Methylated dextran, obtained as a white powder, $[\alpha]_D$ + 214° (in chloroform), OMe 44·5% (maximum attainable), was fractionated by addition of light petroleum to a chloroform solution and the properties of the fractions served to demonstrate its essential homogeneity. It showed considerable resistance to the usual hydrolytic reagents and for its complete hydrolysis recourse was made to the reagent usually employed for the hydrolysis of methylated cellulose, *viz.*, 50% aqueous acetic acid containing 4% of concentrated hydrochloric acid.

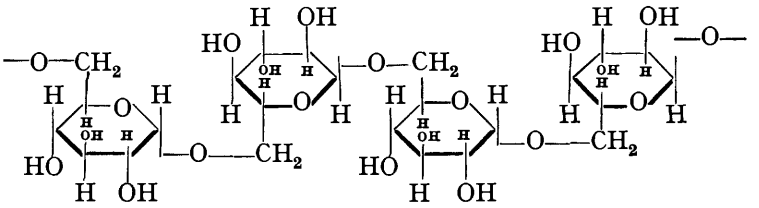
The mixture of methylated sugars resulting from the hydrolysis was separated by fractional distillation of the methylglucosides in a high vacuum : the chief product of hydrolysis, constituting some 90% of the whole, was 2 : 3 : 4-trimethyl glucopyranose. This separated during the distillation of the glucosides as the crystalline trimethyl β-methylglucoside (m. p. 93—94°), which did not depress the m. p. of an authentic specimen. The constitution of the sugar was confirmed by oxidative methods. With nitric acid, trimethyl saccharic acid was formed; it was characterised as 2 : 3 : 4-trimethyl saccharo-

lactone methyl ester, m. p. 106—107° (alone or in admixture with an authentic specimen). Furthermore, the trimethyl sugar was oxidised by bromine to give 2 : 3 : 4-trimethyl δ-gluconolactone. 2 : 3 : 4-Trimethyl glucose readily forms a crystalline *anilide*, m. p. 145—146°.

The lower-boiling fractions obtained in the separation of the glucosides were contaminated with methyl lævulate, which was characterised as the phenylhydrazone, m. p. 106°. It was found necessary to remove this constituent by heating an aqueous solution of these fractions with barium hydroxide, and then to redistil the glucosides in a high vacuum. Ultimately a syrupy first fraction was obtained which had $n_D^{18°}$ 1·4545, an indication of the presence of a small amount of tetramethyl methylglucoside. It was hydrolysed with hydrochloric acid and the syrup obtained (OMe, 46·0%) was treated with aniline. Crystallisation of the product from alcohol yielded tetramethyl glucopyranose anilide (m. p. 135—136°) identical with a specimen prepared from tetramethyl glucose.

The higher-boiling fractions (10% of the whole) obtained in the distillation of the glucosides had the composition of a dimethyl methylhexoside. Since on methylation of these fractions and hydrolysis of the product, only tetramethyl glucopyranose was obtained, it is evident that the dextran is constituted entirely of glucose units.

The isolation of 2 : 3 : 4-trimethyl glucose in 90% yield from the methylated dextran shows the dextran to be constituted on the same general plan as starch, cellulose, and glycogen, in that the molecule is a chain composed of *d*-glucopyranose units linked glucosidically with each other. In the dextran, however, the linkages involve the terminal primary alcohol group of each glucose unit and not the hydroxyl group on $C_4$ as is the case with starch or cellulose. The configuration of this 1 : 6-glucosidic link in the dextran cannot at present be determined with certainty, but there are indications, in particular the high positive rotation (+ 180°) of the dextran and its downward mutarotation during hydrolysis, which suggest that the 1 : 6-linkage has the α-configuration. The main chain of the dextran will therefore be represented by :



Furthermore, the isolation of tetramethyl glucose, albeit in small yield, makes possible a decision between the alternative structures for the dextran molecule of a closed loop of glucopyranose units and a terminated open chain. The tetramethyl glucose represents an end-group and could be formed if the dextran had a continuous chain structure. The proportion of tetramethyl glucose is minute (0·23% of the weight of methylated dextran) and its presence was detected mainly because of the fortunate circumstance that we were able to separate the anilides by fractional crystallisation on a microscale. The yield of tetramethyl glucose anilide obtained corresponded to a chain length of 550 glucose units in the dextran molecule, but this figure represents a maximum and the real value is probably lower. Control experiments on the degree of separation attainable by fractional crystallisation of mixtures of the anilides of trimethyl glucose and tetramethyl glucose indicated that, although qualitative identification of the " tetra " compound in amounts constituting 10% of the mixture could readily be obtained, a quantitative separation of this material was not achieved. It is considered, therefore, that the value of 200 units obtained by osmotic pressure measurements (Carter and Record, *Chem. and Ind.*, 1936, **55**, 218) on the methylated dextran gives a more trustworthy value for the minimum chain length of methylated dextran.

The proof of structure of the dextran of *Leuconostoc dextranicum* presented here was published in a preliminary note (*Nature*, 1938, **141**, 876). Subsequently Fairhead, Hibbert, and Hunter (*Canadian J. Res.*, 1938, **16**, 151) published an account of an investigation

which confirmed our main findings. An " end " unit was not, however, detected by these authors, who therefore envisaged a closed loop structure for the dextran.

## Experimental.

The dextran was prepared and purified by Stacey and Youd's method (*Biochem. J.*, 1938, **32**, 1943). It was a white, non-reducing, granular powder, soluble in cold water with the formation of viscous, faintly opalescent solutions: $[\alpha]_D^{20°}$ + 180°(*c*, 1·1); ash, 0·4%; N, 0·3%. On hydrolysis with N-sulphuric acid, crystalline glucose in 92% yield was isolated and from the small amount of non-crystalline residue, glucose phenylosazone was prepared. It is reasonable, therefore, to regard the polysaccharide as constituted entirely of glucose units.

*Methylation.*—The dextran (10 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.). Acetone in portions of 50 c.c. was added at intervals. At the end of the methylation (4 hrs.) the acetone was boiled off, and the solution neutralised with 5N-sulphuric acid. Partially methylated dextran separated from the hot solution as a flocculent white powder, which was collected on muslin. Three methylations gave a product (OMe, 37—38%) which appeared to be insoluble in all solvents. It swelled, however, in contact with dioxan and use was made of this property to facilitate the subsequent methylations. After 12 methylations the product (8 g.) had OMe, 44·0% and $[\alpha]_D^{20°}$ + 210° in chloroform (*c*, 1·2). This material (70 g.) was prepared and fractionated, as shown below, by the graded addition of light petroleum to a chloroform solution.

| Fraction. | % Yield. | $[\alpha]_D^{21°}$. | % OMe. | % Ash. | $\eta_{sp.}$ in *m*-cresol. |
|---|---|---|---|---|---|
| 1 | 19·1 | +215° | 43·7 | 1·47 | 0·53 |
| 2 | 31·4 | +210 | 44·6 | 0·06 | 0·49 |
| 3 | 40·2 | +214 | 44·5 | 0·14 | 0·51 |
| 4 | 9·3 | +196 | 43·6 | 0·19 | 0·35 |

Fractions 1 and 4 were remethylated until the methoxyl content was 44·5%.

*Hydrolysis of Methylated Dextran.*—The compound (55 g.) was dissolved in 50% aqueous acetic acid (1200 c.c.) containing 4% of concentrated hydrochloric acid and heated at 95° until the specific rotation reached an equilibrium value $[\alpha]_D^{20°}$ + 58°, calculated on original material (9 hours). The solution was concentrated at 20° in a vacuum to a syrup, which was dissolved in water (500 c.c.). The acid was neutralised with calcium carbonate, and the solution filtered. The clear filtrate was extracted several times with chloroform, and the extracts dried over anhydrous magnesium sulphate and concentrated to a syrup (A) (24·5 g.). The aqueous solution was evaporated to dryness, and the residue extracted with boiling chloroform. These extracts yielded a second syrup (B) (28 g.).

The fractions (A) and (B) were separately converted into the methylglucosides by boiling with methyl-alcoholic hydrogen chloride (2%). The glucosides, isolated in the usual way, were then distilled in a high vacuum from a Widmer flask. In a preliminary fractionation of (A) a low-boiling mobile syrup, b. p. (bath temp.) 70—75°, $n_D^{20°}$ 1·4225, OMe 25·8%, was collected. This was mainly methyl lævulate (OMe, 23·8%), which was characterised as the phenylhydrazone, m. p. 106° (alone or in admixture with an authentic specimen). The remainder of fraction (A) was dissolved in water and treated at 60° for 2 hours with a saturated solution of barium hydroxide in order to remove any methyl lævulate. The solution was concentrated, and the glucosides extracted in the usual way and fractionally distilled in a high vacuum.

From (A) the following main fractions were isolated:

| Fraction. | B. p. (bath temp.) at 0·01 mm. | Yield, g. | $n_D^{18°}$. | % OMe. | Physical state. |
|---|---|---|---|---|---|
| 1st drop | 80° | — | 1·4522 | — | — |
| 1a | 82—90 | 0·86 | 1·4545 | 52·3 | Colourless syrup |
| 2a—5a | 112—125 | 16·0 | 1·455—1·457 | 50—51 | Crystalline solid |
| 6a | Residues | 7·5 | — | — | Viscid syrup |

From (B) the following were isolated:

| | | | | | |
|---|---|---|---|---|---|
| 1b | 124—125 | 23·6 | 1·4560 | 48 | Crystalline solid |
| 2b | Residues | 5·0 | — | — | Viscid syrup |

The residues 6a and 2b were rehydrolysed with 50% glacial acetic acid containing 4% of hydrochloric acid for 5 hours and the products were isolated as before and treated with barium

hydroxide solution to remove methyl lævulate. Isolation and distillation of the glucosides gave the following fractions :

| Fraction. | B. p. (bath temp.) at 0·01 mm. | Yield, g. | $n_D^{20°}$. | % OMe. | Physical state. |
|---|---|---|---|---|---|
| 1R—5R | 118—125° | 8·0 | 1·458—1·459 | 49—59 | Crystalline |
| 6R | 125—130 | 0·4 | 1·4630 | 43·4 | Viscid syrup |
| 7R | 140—150 | 1·0 | 1·4700 | 41·7 | ,, ,, |
| 8R | 150 | 1·4 | 1·4720 | 39·0 | ,, ,, |

*Identification of Tetramethyl Glucose in Fraction* 1*a*.—Fraction 1a had $[\alpha]_D^{21°}$ + 14·0° in water (*c*, 1·0) (Found : C, 51·6; H, 8·2; OMe, 52·3. Calc. for $C_{10}H_{20}O_6$ : C, 50·8; H, 8·5; OMe, 52·5%. Calc. for $C_{11}H_{22}O_6$ : C, 52·8; H, 8·8; OMe, 62·0%). Hydrolysis was carried out with 6% hydrochloric acid at 95° and the following changes were observed : $[\alpha]_D^{20°}$ + 13·0°(initial); + 17°(25 mins.); + 45°(55 mins.); + 65°(85 mins.); + 75°(145 mins.); + 78°(205 mins.); + 80°(500 mins.), equilibrium value. The hydrolysis solution was neutralised with barium carbonate and evaporated to dryness, and the sugar was extracted with chloroform, removal of which left a colourless syrup having $[\alpha]_D^{20°}$ + 82° in water (*c*, 1·2) (Found : OMe, 46·0. Calc. for $C_9H_{18}O_6$ : OMe, 41·9%). Treatment of the sugar with aniline in boiling alcoholic solution gave an anilide, which was fractionally crystallised from absolute alcohol. The anilide which separated was tetramethyl glucopyranose anilide; yield, 20% (Found : OMe, 40·3. Calc. for $C_{16}H_{25}O_5N$ : OMe, 40·0%). It had m. p. 135—136°, not depressed by an authentic specimen prepared under identical conditions from tetramethyl glucopyranose. The amount of tetramethyl glucose was estimated to be 0·23% of the weight of methylated dextran, corresponding to a chain length of 550 glucose units.

*Identification of the Trimethyl Methylhexoside Fractions*.—The crystalline material was drained for several days on a porous tile and after recrystallisation from ether–light petroleum had m. p. 93—94° (alone and in admixture with 2 : 3 : 4-trimethyl β-methylglucopyranoside) and $[\alpha]_D^{20°}$ − 21° in water (*c*, 1·0) (Found : C, 50·8; H, 8·5; OMe, 52·2. Calc. for $C_{10}H_{20}O_6$ : C, 50·8; H, 8·5; OMe, 52·5%). The glucoside was hydrolysed with hydrochloric acid (6%) at 100°, and the sugar isolated in the usual way. After distillation at bath temp. 147—150°/0·01 mm. it showed $n_D^{19°}$ 1·4690 and $[\alpha]_D^{20°}$ + 60° (*c*, 1·0) (Found : OMe, 41·2. Calc. for $C_9H_{18}O_6$ : OMe, 41·9%). The sugar, 2 : 3 : 4-trimethyl glucose (0·1 g.), was converted into the *anilide* (0·13 g.), which was recrystallised from ether–light petroleum. It had m. p. 145—146° (Found : C, 58·8; H, 7·3; OMe, 29·3; N, 4·4. $C_{15}H_{23}O_5N$ requires C, 58·6; H, 7·5; OMe, 30·2; N, 4·6%). The porous tile was extracted with boiling chloroform, removal of which left a syrup, which again partly crystallised. A portion (0·5 g.) was methylated three times with silver oxide and methyl iodide, giving a syrup (0·54 g.), b. p. 95—100°/0·01 mm., $n_D^{23°}$ 1·4435, OMe, 60·6%. Hydrolysis with hydrochloric acid at 100° yielded 2 : 3 : 4 : 6-tetramethyl glucopyranose (0·49 g.), m. p. 83—84°. A further amount of the syrupy trimethyl methylglucoside was hydrolysed with hydrochloric acid (6%), showing $[\alpha]_D$ + 113° ⟶ + 68° in 9 hours (*c*, 2·2). The product was isolated in the usual way and converted into the anilide, m. p. 145—146°, identical with 2 : 3 : 4-trimethyl glucopyranose anilide obtained as above.

*Oxidation of* 2 : 3 : 4-*Trimethyl Glucose to* 2 : 3 : 4-*Trimethyl* δ-*Gluconolactone*.—Oxidation of the sugar (2·6 g.) with bromine at 40° gave a lactone (1·97 g.), b. p. 125—135°/0·01 mm., $n_D^{20°}$ 1·4688 (Found : OMe, 41·9. Calc. for $C_9H_{16}O_6$ : OMe, 42·2%). The lactone showed the following changes in water : $[\alpha]_D^{20°}$ + 80·4°(initial), 72°(30 mins.), 66°(65 mins.), 61°(100 mins.), 52°(140 mins.), 49°(180 mins.), 42°(240 mins.), 39°(300 mins.), 32°(720 mins.) (equilibrium value).

*Oxidation of* 2 : 3 : 4-*Trimethyl* δ-*Gluconolactone*.—The lactone (1·2 g.) was dissolved in nitric acid (*d* 1·26; 20 c.c.) and heated for 1½ hours at 90—100°. The acid was removed by distillation in steam, the oxidation product was boiled with 1·5% methyl-alcoholic hydrogen chloride, and the esters formed were distilled in a high vacuum. Two fractions were isolated : I, b. p. 100°/0·012 mm., $n_D^{22°}$ 1·4370 (0·15 g.); II, b. p. 140°/0·01 mm., $n_D^{22°}$ 1·4480 (0·67 g.). Fraction II crystallised and was identified as 2 : 3 : 4-trimethyl saccharolactone methyl ester, m. p. and mixed m. p. 106—107°, $[\alpha]_D^{17°}$ + 31° (equilibrium value after 30 hours).

*Examination of the Dimethyl Methylhexoside Fractions*.—A sample from fraction 7R (0·5 g.) was methylated three times with silver oxide and methyl iodide to tetramethyl methylglucoside, which was then hydrolysed to tetramethyl glucopyranose, m. p. 84°, the yield (0·4 g.) showing that the dimethyl fractions were glucose derivatives.

Dimethyl methylglucoside (8R) (0·5 g.) was hydrolysed with 6% hydrochloric acid ($[\alpha]_D$ + 105° ⟶ + 77° in 5 hours), and the resultant dimethyl glucose (0·4 g.) converted into the *anilide* (0·2 g.), m. p. 172—173° (Found : OMe, 19·5. $C_{14}H_{21}O_5N$ requires OMe, 21·1%).

Further investigations revealed that the dimethyl glucoside fraction was a mixture—a satisfactory separation of which has not yet been accomplished.

*Examination of Artificial Mixture of Methylated Glucose Anilides.*—The separation of 2 : 3 : 4 : 6-tetramethyl glucose anilide in admixture with 2 : 3 : 4-trimethyl glucoside anilide in various proportions was attempted. A typical experiment is as follows: 2 : 3 : 4-Trimethyl glucopyranose (80 mg.) and 2 : 3 : 4 : 6-tetramethyl glucopyranose (20 mg.) were heated with the equivalent weight of aniline in boiling ethyl alcohol for 6 hours. On removal of the alcohol a crystalline mass remained. This was dissolved in the minimum amount of hot ethyl alcohol and kept at 0°. The total crop of crystalline material (7 mg.) which separated was 2 : 3 : 4 : 6-tetramethyl glucopyranose anilide. A quantitative recovery of the total amount of tetramethyl glucopyranose anilide was not obtained and, on a micro-scale, 10% concentration was the lowest at which this material was conveniently separated. Similar results were obtained with 2 : 3 : 6-trimethyl glucose.

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