cedure outlined for the separation of small quantities of partially methylated glucosides whereby an almost quantitative recovery (95–97%) can be effected.

The glucosides can be separated in a high degree of purity and with accompaniment of only very small intermediate fractions.

The amount of non-volatile residue formed was never more than one per cent.

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[CONTRIBUTION FROM THE DIVISION OF INDUSTRIAL AND CELLULOSE CHEMISTRY, MCGILL UNIVERSITY]

Studies on Reactions Relating to Carbohydrates and Polysaccharides. LXVI. Structure of the Dextran Synthesized by the Action of *Leuconostoc Mesenteroides* on Sucrose[1]

BY IRVING LEVI, W. LINCOLN HAWKINS AND HAROLD HIBBERT

In a previous communication[2] an investigation of the structure of dextran synthesized by the action of *L. mesenteroides* on sucrose was described. Hydrolysis of the trimethyl dextran by the action of methanolic hydrogen chloride yielded dimethyl, trimethyl and tetramethyl methyl glucosides in the approximate ratio of 1:3:1. The products of hydrolysis were identified as 2,3-dimethyl methyl glucoside, 2,3,4-trimethyl methyl glucoside and 2,3,4,6-tetramethyl methyl glucoside. Based on these results a branched chain structure for the dextran was proposed.

These results were subsequently criticized by Brauns[3] on the following grounds: (a) the dextran was incompletely methylated; (b) the ratio of tetra- to tri- to dimethyl methyl glucosides of 1:3:1 was not conclusive because of the inefficient fractional distillation employed; and (c) the large percentage (18.4%) of material lost during fractionation. A re-investigation of this dextran was therefore made in order definitely to establish its structure.

Discussion of Results

Most complex polysaccharides such as mannan,[4,5] glycogen,[6,7,8] and araban[9] contain intricately branched chains, every branching position of which yields a dimethyl methyl glycoside in the case of hexosans and a monomethyl methyl glycoside in the case of pentosans. In methylation studies of such polysaccharides a final methoxyl value of one or two per cent. lower than the theoretical can render the results worthless with respect to the extent of branching and so preclude an accurate structural assignment. The great importance of complete methylation of polysaccharide products prior to structural determination by hydrolysis cannot be over-stressed and has been all too frequently neglected[10,11] with the result that conclusions drawn have only a restricted value.

Throughout this investigation every precaution was taken to obtain yields of material as nearly quantitative as possible in order that the results obtained could be based on a very high percentage of the starting material and therefore be of true significance. With dextran and its derivatives this involved reducing experimental manipulations and transfers to a minimum since these compounds are very difficult to handle because of their physical properties.

Dimethyl sulfate and alkali yielded a partially methylated dextran (40–41% OCH_3) which was further methylated to the theoretical value of 45.6% OCH_3 (calcd. for $C_6H_7O_2(OCH_3)_3$) in 71.4% over-all yield by a modified Muskat technique.[12] Hydrolysis of the completely methylated dextran was carried out with methanol–hydrogen chloride, in sealed glass bombs, heated to 140–142° for sixty to sixty-five hours in a tilting electric oven. The resulting glucosidic mixture, obtained in 95% yield, was fractionated by the new technique described in the preceding communication[13] using the modified Podbielniak column,[14] and an excellent separation of the glucosides effected with an over-all recovery of 97%. Of the 5% lost during

(1) Original manuscript received August 13, 1941.
(2) Fowler, Buckland, Brauns and Hibbert, *Can. J. Research*, **B15**, 486 (1937).
(3) Brauns, *ibid.*, **B16**, 73 (1938).
(4) Haworth, Hirst and Isherwood, *J. Chem. Soc.*, 784 (1937).
(5) Haworth, Hirst, Isherwood and Jones, *ibid.*, 1878 (1939).
(6) Bell, *Biochem. J.*, **30**, 1612, 2144 (1936).
(7) Bell, *ibid.*, **31**, 1683 (1937).
(8) Haworth and Isherwood, *J. Chem. Soc.*, 577 (1937).
(9) Hirst and Jones, *ibid.*, 496 (1938).
(10) Freudenberg, *Ber.*, **69**, 2043 (1936).
(11) Haworth, Raistrick and Stacey, *Biochem. J.*, **29**, 612 (1935).
(12) Muskat, THIS JOURNAL, **56**, 693, 2448 (1934).
(13) Levi, Hawkins and Hibbert, *ibid.*, **64**, 1957 (1942).
(14) Podbielniak, *Ind. Eng. Chem., Anal. Ed.*, **3**, 177 (1931); *ibid.*, **5**, 119 (1933).

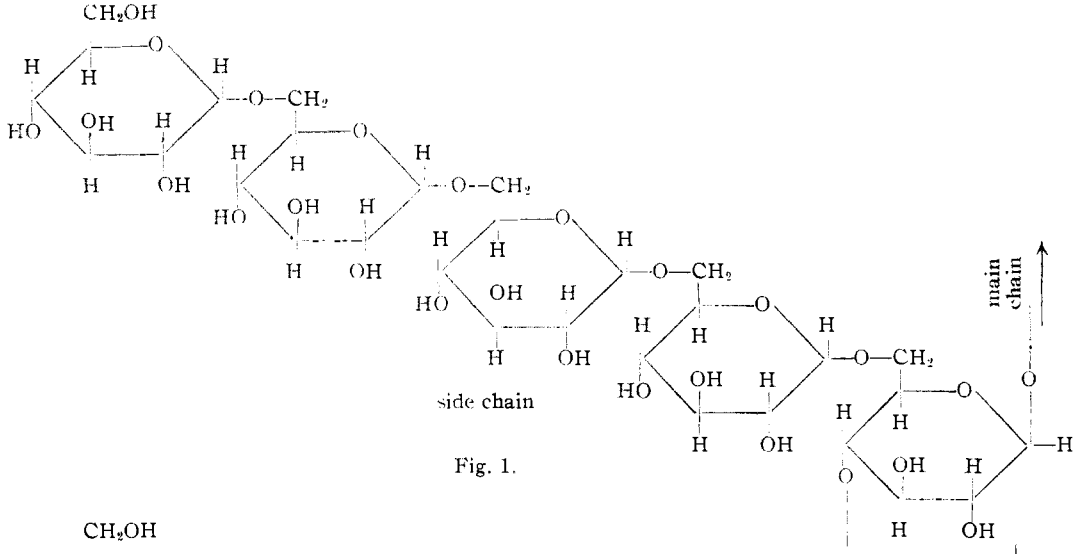

Fig. 1.

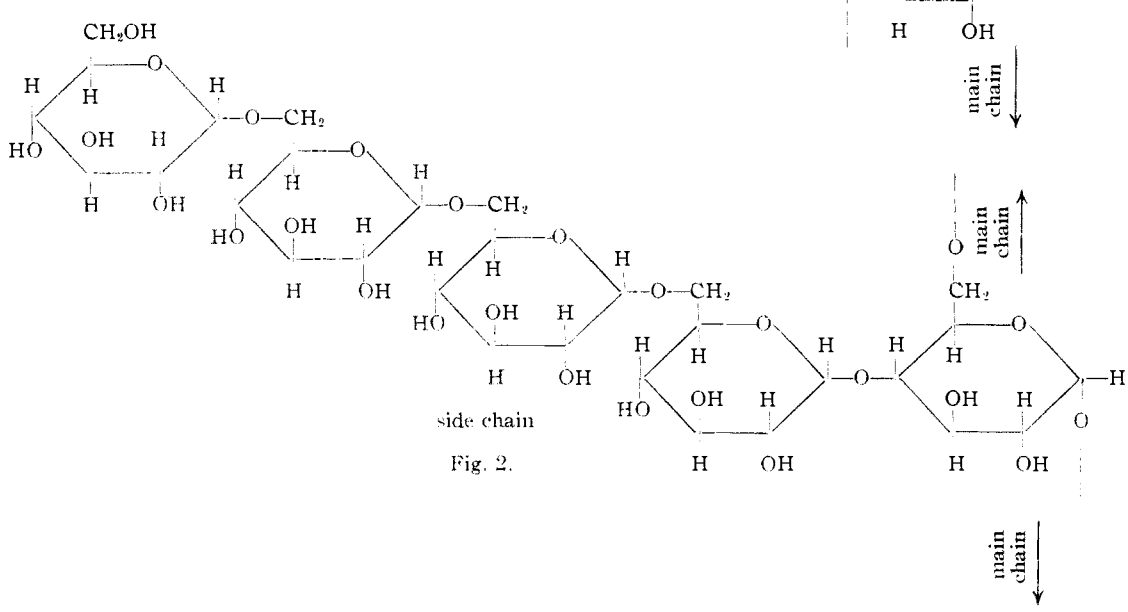

Fig. 2.

hydrolysis, 4% could be accounted for as methyl levulinate. It was shown by control experiments that the three partially methylated monosaccharides obtained from the hydrolysis of methylated dextran were all decomposed in this manner and to the same extent (1.5%) so that this side reaction does not affect the final ratio. By this procedure the ratio of tetra- to tri- to dimethyl methyl glucoside was established as exactly 1:3:1, thus verifying the earlier results of Hibbert and co-workers.[2]

The tetramethyl methyl glucoside and trimethyl methyl glucoside were identified as 2,3,4,6-tetramethyl methyl glucoside and 2,3,4-trimethyl methyl glucoside, respectively.

The dimethyl methyl glucoside was identified as the 2,3-derivative by oxidation of the dimethyl glucose to the corresponding dimethyl gluconic acid (84% yield) by the method of Hudson and Isbell.[15]

The dimethyl gluconic acid was characterized as the crystalline 2,3-dimethyl gluconophenylhydrazide (81% yield).

Based on the above evidence, two tentative formulas for dextran (Figs. 1 and 2), differing only in the position of attachment of the side chain, are suggested. These, however, do not exhaust all the possibilities and it is conceivable that the side chain may consist of three, two or even one unit with a corresponding lengthening of the primary chains.

Three of the linkages between the building units are of the 1,6 type while the remaining two are

(15) Hudson and Isbell, THIS JOURNAL, **51**, 2225 (1929).

either 1,4 or 1,6. No decision can be reached at present as to whether the linkages are α or β.

Experimental

Methylation of Dextran.—Thirty grams of dextran was suspended in 300 cc. of water, made alkaline by the addition of 25 cc. of 30% sodium hydroxide and methylated by the Haworth method in an atmosphere of nitrogen, using ten successive portions of 100 cc. of sodium hydroxide and 40 cc. of dimethyl sulfate. The alkali and the dimethyl sulfate were added to the reaction mixture concurrently. The complete reaction was carried out at room temperature.

The sodium sulfate formed during the methylation was removed by dialysis through a cellophane membrane. Three such methylations were carried out; yield, 34.0 g.; OCH_3, 40.5%.

Further methylations were effected using an original modification of Muskat's method.[12] The apparatus consisted of a cylindrical Pyrex container (7.5 × 22.5 cm.) with a side arm for the passage of ammonia. A condenser and mercury-sealed stirrer were attached to the flask by ground glass joints.

Thirty-four grams of dextran (OCH_3, 40.5%) was suspended in anhydrous anisole (340 cc.) in the reaction flask and 40 cc. of anisole distilled off under reduced pressure (20 mm.) to remove the last traces of water. The remaining dextran–anisole suspension (300 cc.) was stirred overnight. The anisole was then frozen by means of a chloroform–solid carbon dioxide-bath, and metallic sodium (0.9 g.) added to dry the ammonia (200 cc.), which was condensed on the solid anisole (time three to four hours). Metallic sodium (4 g.) was added, the anisole allowed to melt, and the mixture stirred for three to four hours. The ammonia was allowed to evaporate spontaneously. Twenty cc. of anisole was distilled off under vacuum and in an atmosphere of nitrogen, to remove the last traces of ammonia, methyl iodide (100 g.) added and the mixture refluxed overnight at 60° on the water-bath. Additions of both sodium and methyl iodide were made through the side-arm by temporarily removing the condenser.

The methyl iodide was removed by distillation under reduced pressure through a distilling head which fitted the reaction flask (ground glass joints throughout). Twenty cc. of anisole was added and then removed by vacuum distillation in order to expel traces of moisture.

This procedure was repeated twice more without removing the product from the flask (trial experiments had shown that the inorganic impurities did not interfere with the methylation).

After the third methylation there was considerable inorganic material present which was removed at this stage as follows. The reaction mixture was taken to dryness (20 mm.) in an atmosphere of nitrogen, water (300 cc.) added, the mixture heated, with occasional stirring, to the boiling point and kept at this temperature for ten minutes. The dextran was removed by centrifuging the cooled solution, then dried in the vacuum oven overnight at 55–60°. The well-dried, partially-methylated dextran dissolved readily in cold chloroform (10 cc. of chloroform per gram of methylated dextran) and any appreciable residue was removed by centrifuging the chloroform solution, followed by filtration. One to two volumes of 30–50° petroleum ether was added until an appreciable turbidity was apparent. This initial slight precipitate contained much of the residual ash. The clear filtrate was precipitated into 30–50° petroleum ether (total volume twenty times that of chloroform used), and the resulting precipitate washed twice with fresh 30–50° petroleum ether and dried [55° (20 mm.)] for thirty hours; yield, 29.8 g.; OCH_3: 1st Muskat methylation 41.5%; 2nd, 42.3%; 3rd, 44.0%. (The methoxyl values were obtained by removing and purifying a small representative sample.) The dextran was then methylated three more times as above and purified in the same manner; yield, 27.6 g.; OCH_3: 4th Muskat methylation 44.9%; 5th, 45.3%; 6th, 45.6%. Calcd. for: $C_6H_7O_2(OCH_3)_3$: C, 52.94; H, 7.84; OCH_3, 45.6. Found: C, 52.70; H, 7.92; OCH_3, 45.6.

Hydrolysis of Methylated Dextran.—Fully methylated dextran (7.100 g.) was hydrolyzed in three separate portions of 3.060, 1.520 and 2.520 g., respectively.

In a typical hydrolysis 3.060 g. of methylated dextran was suspended in anhydrous methanol (60 cc.) containing 2% hydrogen chloride, sealed in a glass bomb-tube and heated at 140–142° in a tilting electric oven for 60–65 hours. After this time the dextran had dissolved completely and the solution changed from a pale yellow to a clear reddish brown color.

The bomb was cooled to 0°, opened, and the excess hydrogen chloride gas allowed to escape while the solution attained room temperature. The open end of the bomb was fitted, during this period, with a Kjeldahl trap as a precaution in case of a too vigorous effervescence.

The solution was transferred to a centrifuge cup and neutralized (litmus) with silver carbonate, the insoluble silver salts removed by centrifugence and filtration and washed with three portions (15 cc. each) of anhydrous methanol, the washings being added to the original filtrate. At this point the methanolic solutions from the three separate hydrolyses were combined and the solvent removed under reduced pressure [20 mm. (50°)].

The resulting sirup was dissolved in 142 cc. of water so that a 10-cc. aliquot contained the glucosides originating from 0.5 g. of methylated dextran. To 10 cc. of this solution was added 50 cc. of 2,4-dinitrophenylhydrazine (containing 0.2 g. of reagent). After twenty minutes the precipitate (A) was filtered, dried under suction and finally in a vacuum desiccator; yield, 0.030 g. representing 4.2% decomposition of the dextran; recrystallized from methanol; melting point, 138–139°. A mixed melting point with an authentic sample of 2,4-dinitrophenylhydrazone of methyl levulinate showed no depression.

To the filtrate (B) an additional 50 cc. of reagent was added and the solution allowed to stand at room temperature for two and one-half hours. No further precipitate formed.

Removal of Methyl Levulinate.—To the remainder of the aqueous solution containing the glucosides from 6.6 g. of methylated dextran was added a barium hydroxide solution (20 cc.), saturated at 92–93°, and the mixture kept at 60–62° on the water-bath for two hours. It was then taken to dryness [20 mm. (50–60°)], and the residue extracted by hand shaking with the following successive portions of hot chloroform: 100, 50, 50, 50, 50, 25, 25, 25 cc.

TABLE I

FRACTIONATION OF GLUCOSIDIC MIXTURE OBTAINED FROM HYDROLYSIS OF FULLY-METHYLATED DEXTRAN

Fraction	Physical state	Fraction, g.	OCH_3, %	"Tetra," g.	"Tri," g.	"Di" g.
1	Colorless sirup	1.204	61.0	1.204		
2	Colorless sirup and white crystals	0.207	57.8	0.113	0.094	
3	White crystals (pure 2,3,4-trimethyl methyl glucoside)	0.605	52.8		0.605	
4	White crystals and colorless sirup (α and β mixture)	2.811	52.4		2.811	
5	Colorless sirup	0.397	46.5		0.172	0.225
6	Light yellow sirup	1.013	42.0			1.013
Total				1.317	3.682	1.238
Ratio				1.01	3.00	1.07

The theoretical methoxyl values for tetra-, tri-, and dimethyl methyl glucosides are 62.0, 52.6, and 41.9%, respectively. The amounts of each present in the small intermediate fracrions (2 and 5, Table I) were calculated on this basis.

The combined extracts were evaporated at 40° and 20 mm. pressure, and the residue distilled under high vacuum (0.005 mm.) to give a clear sirup; yield, 7.245 g. (95%). Calcd. for $C_6H_8O_2(OCH_3)_4$: OCH_3, 52.6%; found, OCH_3, 52.3%.

Fractionation of the Glucosides.—The glucosidic mixture (6.43 g.) was fractionated using the same conditions and technique as described in the preceding paper.[13] The results of the fractionation, methoxyl analyses and allocation of the two intermediate fractions among the glucosides are summarized in Table I.

Identification of the Glucosides

(a) **Tetramethyl Methyl Glucoside.**—A portion (0.70 g.) of Fraction 1 was hydrolyzed with 15 cc. of 5% sulfuric acid at 100° for eighteen hours, after which time the rotation was constant.

The acid solution was neutralized to litmus with solid barium carbonate, filtered, and the filtrate taken to dryness [55° (20 mm.)], in an atmosphere of nitrogen. The residue was extracted with four 25-cc. portions of hot anhydrous acetone, the combined acetone solutions filtered, and the solvent removed at 20° leaving a pale yellow sirup, to which 10 cc. of anhydrous ether was added. On standing a precipitate of fine white needles was obtained as the ether evaporated; weight 0.50 g. (75% of theoretical). The product was recrystallized from low-boiling (30–50°) petroleum ether containing 5% diethyl ether; m. p. 90–91°. A mixed melting point with an authentic sample of 2,3,4,6-tetramethyl glucose showed no depression.

(b) **Trimethyl Methyl Glucoside.**—A portion (0.58 g.) of Fraction 3 was recrystallized from low boiling (30–50°) petroleum ether containing 5% diethyl ether. A mass of fine white needles was obtained (0.42 g.), m. p. 93–94°, which showed no mixed melting point depression with an authentic sample of 2,3,4-trimethyl-β-methyl glucoside.

(c) **Dimethyl Methyl Glucoside.**—A portion (0.60 g.) of Fraction 6 was hydrolyzed to the corresponding dimethyl glucose (0.538 g.) using the procedure outlined above for the hydrolysis of the tetramethyl methyl glucoside. Calcd. for $C_6H_{10}O_4(OCH_3)_2$: OCH_3, 29.9. Found: OCH_3, 30.2.

The dimethylglucose (0.538 g.) was oxidized to the corresponding dimethylgluconic acid by use of the method of Hudson and Isbell;[15] weight of dimethylgluconic acid, 0.487 g. (84%).

The dimethylgluconic acid (0.487 g.) was characterized as the crystalline 2,3-dimethylgluconophenylhydrazide.[16] The weight of recrystallized product (from ethanol) was 0.556 g. (81.4%), (of fine white needles); m. p. 166.5–167.5° (uncor.); mixed melting point with authentic 2,3-dimethylgluconophenylhydrazide showed no depression. Calcd. for $C_{14}H_{22}O_6N_2$: C, 53.5; H, 7.0; N, 8.9; OCH_3, 19.7. Found: C, 53.5; H, 7.1; N, 9.0; OCH_3, 19.5.

Summary

1. The complete methylation of the dextran, synthesized by *Leuconostoc mesenteroides*, has been accomplished in an over-all yield of 71.4%.

2. Hydrolysis of trimethyl dextran and fractionation of the resulting glucoside mixture, employing quantitative technique, have established the ratio of tetra- to tri- to dimethyl methyl glucoside as 1:3:1.

3. The three glucosides have been identified, and based on these results a tentative formula for the structure of dextran has been proposed.

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(16) Evans, Levi, Hawkins and Hibbert, *Can. J. Research*, forthcoming publication.