Determination of Infrared Spectra.—The infrared spectra were measured over the wave length range of 2 to 16 microns using a Baird Spectrophotometer. The spectra of liquid samples were measured in capillary cells, those of solids as mineral oil mulls.

ST. LOUIS, MISSOURI

[CONTRIBUTION FROM THE NORTHERN UTILIZATION RESEARCH BRANCH[1]]

Preparation of Methyl Glycosides of Homologous α-1,6'-Linked Gluco-oligosaccharides and the Optical Rotation of their Cuprammonium Complexes[2,3]

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Cuprammonium complexes of several native dextrans were found to be much more levorotatory than was expected from the optical rotation of methyl glucopyranoside in cuprammonium solution and the structure of these dextrans indicated by periodate oxidation analysis. To determine the optical activity of cuprammonium complexes of α-1,6'-linked anhydroglucopyranoside units, free from effects of branching, the lowest members of the methyl isomaltoside homologous series were prepared by methanolysis of NRRL B-512 dextran. Eleven members, DP 1 through 11, were chromatographically separated on a cellulose column and were characterized by paper chromatograms, periodate oxidation analysis, tests for furanoside structure, reducing power, methoxyl content, and optical rotation at 4358 Å. in aqueous and cuprammonium solutions. For glycosides of DP greater than unity, the difference between the molecular rotation per anhydroglucose unit in cuprammonium and aqueous solutions was proportional to $(DP - 1)/DP$. From this relationship a value of $-99,000°$ was determined for the molecular rotational shift of an α-1,6'-linked unit in a dextran molecule. It was concluded that the presence of a glucosyl unit on number 6 hydroxyl leads to a marked preference for complex formation at the 2,3-position compared with the 3,4-position.

The change in optical rotation caused by cuprammonium–glycol complexing has been used by Reeves[4] to assign ring conformations to methyl glycopyranosides and to determine qualitatively the predominant type of glycosidic linkage in polysaccharides. Reeves has shown that cuprammonium complexing at the 2,3-hydroxyl positions of methyl 4-methyl-D-glucopyranoside causes a decrease of 200,000° in the molecular rotation, whereas complexing at the 3,4-hydroxyl positions of methyl 2-methyl-D-glucopyranoside produces an increase of 210,000° in the molecular rotation. The molecular rotation of methyl D-glucopyranoside underwent an increase of only 25,000° in cuprammonium solution, presumably because of the compensating effects of complex formation at both the 2,3- and 3,4-positions. In each of the above cases the effect of cuprammonium–glucoside complexing on rotation was found largely to be independent of the anomeric configuration of the glucopyranoside.

Results on the glucopyranosides suggested that information on the glucosidic linkages in dextrans could be gained from the shift in optical rotation occurring on copper complex formation. Our measurements, however, on several native dextrans produced by different strains of *Leuconostoc mesenteroides* showed that dextrans in cuprammonium solution were much more strongly levorotatory than would be expected from the structures indicated by periodate oxidation analysis (see also 4). For example, NRRL B-512 dextran was found by periodate oxidation[5] to contain about 95% unbranched α-1,6'-linked D-anhydroglucopyranoside units, and this has been confirmed by methylation analysis.[6] Because of the availability of the 2-, 3- and 4-hydroxyls, these units would be expected to behave analogously to methyl D-glycopyranoside in the reaction with cuprammonium. The molecular rotation per anhydroglucose unit of this dextran, however, was found to decrease 90,000° in cuprammonium solution instead of increasing about 25,000°. Since the branched units in B-512 dextran have been shown[6] to carry linkages on position 3, these units could not form copper complexes, and, therefore, cannot be responsible for the anomalous rotational shift. It seemed, therefore, that the unbranched α-1,6-linked anhydroglucopyranoside units in dextran must themselves cause a rather large decrease in rotation in cuprammonium solution.

To gain further knowledge of the properties of the cuprammonium complexes of α-1,6'-linked anhydroglucopyranoside units, we prepared the lowest members of the methyl isomaltoside homologous series and determined their molecular rotations in aqueous and in cuprammonium solution. Glycoside derivatives of the oligosaccharides, required in order to confer the alkali stability needed for measurement of optical rotation in cuprammonium solution were obtained by methanolysis of a dextran to produce the methyl isomaltoside homologous series. The smallest members of the series were separated chromatographically on a large cellulose column. Although no one has reported the preparation of a homologous series of oligosaccharides by methanolysis of the parent polysaccharide, the analogous method of hydrolytic degradation is an established procedure. Whistler and Chen-Chuan Tu[7] have isolated the dimer through the

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(2) From a thesis by Troy A. Scott, Jr., submitted to the Graduate School of Bradley University, Peoria, Ill., in partial fulfillment of the requirements for a M.S. degree.

(3) Presented before the Division of Carbohydrate Chemistry at the Meeting of the American Chemical Society in New York, N. Y., September 12–17, 1954.

(4) R. E. Reeves in "Advances in Carbohydrate Chemistry," Vol. 6, edited by W. W. Pigman and M. L. Wolfrom, Academic Press, Inc., New York, N. Y., 1951, p. 107.

(5) A. Jeanes and C. A. Wilham, THIS JOURNAL, **72**, 2655 (1950).

(6) J. W. Van Cleve, W. C. Schaefer and C. E. Rist, Abstracts of the 125th Meeting of the American Chemical Society, March, 1954, p. 8D.

(7) R. L. Whistler and Chen-Chuan Tu, THIS JOURNAL, **74**, 3609 (1952).

hexamer of the β-1,4'-linked xylopyranose series from hydrolyzed xylan. Whelan, Bailey and Roberts[8] have prepared the maltodextrin series up to a *DP* 7. In each of these investigations, a carbon column was used for separating the homologs.

Experimental

Materials and Reagents.—The dextran selected for degradation was produced by the enzymatic action of *Leuconostoc mesenteroides* NRRL B-512 culture filtrate[9,10] liquors on 10% sucrose solution at 22°. Periodate-oxidation analysis of this dextran showed that it contained 96% 1,6'-bonds. Ash and nitrogen contents were 0.03 and 0.02%, respectively.

Chromatographic solvents, *n*-butyl alcohol, pyridine and methyl Cellosolve,[11] were intermediate fractions from distillation of technical-grade materials. Chromatograms on strips cut from 46 × 57-inch sheets of Whatman No. 1 filter paper were developed in a large insulated box, lined with stainless steel. An ammoniacal 10% silver nitrate solution was used for spraying the chromatograms. Whatman cellulose powder was used for chromatographic columns.

Methanolysis of Dextran.—Conditions necessary for obtaining a good yield of oligosaccharides were determined by carrying out small-scale methanolyses in which 1 to 10% suspensions of dextran in 0.2 and 0.5 *N* methanolic hydrogen chloride were refluxed on a steam-bath for periods of time ranging from 1 to 10 hours. Chloride ions were removed from the methanolysis mixture by passage through a column of Duolite A-4 or Duolite A-6 anion-exchange resin in the hydroxyl form. Dowex-1 resin was found to be unsatisfactory for this purpose because of holdup of the glycosides on the column. Similar retention of sucrose has been noted by Phillips and Pollard.[12] The amount of low molecular weight glycosides in each of the methanolyzed samples was determined by quantitative chromatography, using a procedure adapted from that of Dimler, *et al.*[13]

For the large-scale preparation, 100 g. of vacuum-dried dextran was refluxed in 2 liters of methanolic hydrogen chloride (0.22 *N* HCl, 0.05% water) for 6 hours. After cooling, solids were removed from the mixture on a coarse fritted Pyrex filter and were washed thoroughly with reagent grade methanol. Filtrate and washings were deionized by passage through a column of Duolite A-6 anion-exchange resin in the hydroxyl form. A qualitative chromatogram of this methanol-soluble fraction showed that it contained no glycoside of *DP* greater than 5. By anthrone analysis[14] the carbohydrate content of this fraction was found to be 20.0 g., calculated as anhydroglucose. The solution was lyophilized prior to fractionation on a cellulose column.

The residue, insoluble in methanol and remaining on the filter, was dissolved in water and washed through the filter. This solution was neutral in *p*H and contained 79.2 g. of carbohydrate calculated as anhydroglucose from anthrone analysis. After concentration, glycosides in this solution were separated into 5 fractions by successive precipitations at 75, 90, 92.5 and 95% (v./v.) methanol. Chromatograms showed that a relatively high proportion of glycosides of *DP* 10 or less were contained in the subfractions which were soluble in 90% methanol, and these subfractions were combined with the methanol-soluble fraction described above for chromatographic separation on a cellulose column.

Chromatographic Separation of Methyl Glycosides.—For the separation of the methyl glycosides, a section of industrial glass pipe, 4 feet long with an inside diameter of 4 inches, was packed with 3.4 kg. of dry Whatman ashless cellulose powder to a height of 105 cm. The column was provided with a constant-head device connected to a 4-liter reservoir to permit continuous operation without attention. To keep the upper portion of the cellulose well packed,[15] a perforated stainless steel disk, 3.75 inches in diameter, was placed on the surface of the cellulose, and a stainless steel weight provided with 3 legs was laid on the disk. Eluate was collected in a 200-tube, time-controlled, fraction cutter. After the column had been prewashed with 105 liters of water, the effluent contained 3.8 mg. of carbohydrate per liter by anthrone test, with very little decrease in concentration in the last 50 liters. Successive washings then were made with 12 liters of 20% ethanol, 2 liters of 50% ethanol, and finally with the developing solvent, a mixture of *n*-butyl alcohol, methyl Cellosolve and water (BMW) in the ratios 2:1:1. Yellow color appeared in the first BMW eluate; this eluate also contained considerable water-insoluble material, but an anthrone test for carbohydrate was negative.

The top 2 inches of cellulose was removed from the column and thoroughly mixed with a water solution of 28.9 g. (as anhydroglucose) of the combined 90% methanol-soluble and absolute methanol-soluble fractions of methanolyzed dextran described above. Water was evaporated from the slurry, first on a steam-bath and then in a vacuum oven set at 70°, and the dried cellulose, on which the glycosides were absorbed, was replaced at the top of the column. Development was begun with BMW (2:1:1).

Seven liters of eluate were collected before an anthrone test showed the presence of carbohydrate. One liter of this eluate was evaporated to dryness and found to contain 18 mg. of water-insoluble material which apparently had been eluted from the cellulose. When carbohydrate appeared in the eluate, it was collected with the automatic fraction cutter, set at 8 minutes per tube, each tube receiving 23 ± 0.5 ml. Glycoside elution was followed by carbohydrate analysis of the contents of selected tubes, by means of an adaptation of the "heat-of-mixing" anthrone method of Viles and Silverman.[16] Seventeen days elapsed before the heptaoside reached the bottom of the column; at this time, the proportions of the partitioning solvent were changed to 3:2:3 to remove the larger glycosides in a shorter time. This caused the appearance of 2 peaks in fractions 7 and 8, presumably because the new solvent pushed forward the trailing portions of these fractions. As a precaution against the possibility that the second peak of fraction 8 was actually the 9-unit glycoside, this fraction was subdivided between the 2 peaks. No prominent peaks appeared after the 11th, but elution was continued until 130 liters had passed through the column. Figure 1 shows the separation attained, and the portion of each fraction which was saved.

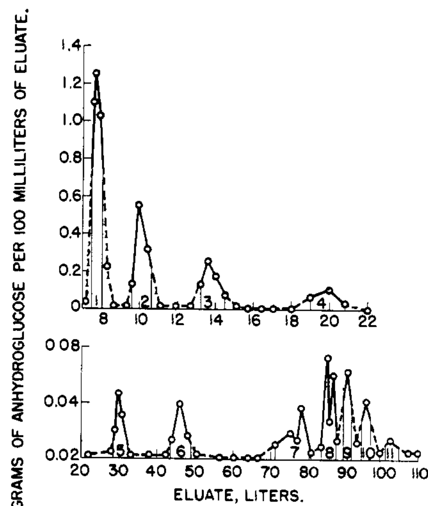

Fig. 1.—Separation of methyl glycosides on cellulose column. Numbers under peaks correspond to fraction numbers and to *DP*'s of methyl glycosides in fractions.

(8) M. J. Whelan, J. M. Bailey and P. J. P. Roberts, *J. Chem. Soc.*, 1293 (1953).

(9) H. J. Koepsell and H. M. Tsuchiya, *J. Bacteriology*, **63** [2], 293 (1952).

(10) V. E. Sohns, S. P. Rogovin, H. F. Conway and C. T. Langford, "Pilot-Plant Production of Clinical-Sized Dextran by Acid Hydrolysis of the Enzymatically Synthesized High Polymer," AIC Bulletin 372, June, 1954.

(11) The mention in this article of firm names or commercial products does not constitute an endorsement of such firms or products by the U. S. Department of Agriculture.

(12) J. D. Phillips and A. Pollard, *Nature*, **171**, 41 (1953).

(13) R. J. Dimler, W. C. Schaefer, C. S. Wise and C. E. Rist, *Anal. Chem.*, **24**, 1411 (1952).

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(15) J. D. Geerdes, Bertha A. Lewis, R. Montgomery and F. Smith, *Anal. Chem.*, **26**, 264 (1954).

(16) F. G. Viles and L. Silverman, *Anal. Chem.*, **21**, 950 (1949).

After concentration in a circulating evaporator at reduced pressure to a volume of approximately 50 ml., each fraction was evaporated to near-dryness at 40°. The volume again was brought to 50 or 100 ml. with water and evaporated to near-dryness. The material then was dissolved in water, diluted to a known volume, and analyzed for carbohydrate. Solutions of the higher glycosides were cloudy at this stage, probably as a result of water-insoluble material removed from the cellulose. Centrifugation at 2800 × gravity cleared the solutions without loss of carbohydrate. Solutions were passed through columns of ion-exchange resins, Dowex-50 and Duolite A-6, to remove any ash eluted from the column and then were lyophilized 6 times to remove traces of *n*-butyl alcohol or methyl Cellosolve. Yields of the glycosides recovered in the dry state as well as the amount of carbohydrate in the portions discarded from each fraction are given in Table I. Of the 28.9 g. placed on the column, 26.5 g. was recovered, representing a 92% recovery. Most of the loss probably occurred during absorption of the original mixture on cellulose and its transfer to the column.

TABLE I

YIELDS OF METHYL GLYCOSIDES SEPARATED ON CELLULOSE COLUMN

Fraction number	Carbohydrate, g. (as anhydroglucose) Retained	Discarded	Accumulated l. eluted at max. concn. Obsd.	Calcd.
1	5.4	1.1	7.6	(7.6)
2	3.8	0.6	9.9	(9.9)
3	2.4	.4	13.6	(13.6)
4	1.6	.3	19.9	19.7
5	1.0	.2	29.7	29.7
6	1.1	.4	45.7	46.0
7	1.6	.1	75.0	72.6
8A	1.0	..	84.5	116.0
8B	0.7	0.3	85.3	
9	.9	.5	90.0	
10	.6	.4	94.3	
11	.4	1.0	101.0	
	20.5	6.0		

Refractionation of Glycosides.—Fractions 1 through 7 gave single, well-defined spots on chromatograms developed with BMW (4:2:3) or butanol-pyridine-water (BPW) in the ratios 1:1:1. Chromatograms of the glycosides of DP greater than 7 were streaked from the starting line, suggesting the presence of carbohydrate of higher molecular weight. The two subfractions of fraction 8 had identical R_f values and were combined. For additional purification, fractions 6, 8 and 10 were combined and passed through a column, 60 cm. long and 75 mm. in diameter. Fractions 7, 9 and 11 also were combined and reseparated on a similar column. Figure 2 shows the separation achieved with BMW (8:4:5) as the partitioning solvent. Fractions were concentrated and lyophilized as before. Qualitative chromatograms showed little improvement in the purity of fractions of DP greater than 7. Quantitative chromatograms showed that 25% of fraction 8 and 60% of fraction 9 did not move from the starting line.

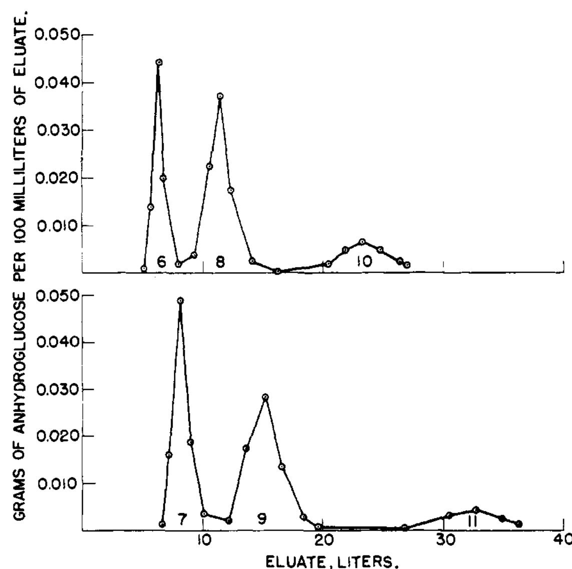

Fig. 2.—Refractionation of methyl glycosides on cellulose column. Numbers under peaks correspond to fraction numbers and to *DP's* of methyl glycosides in fractions.

Characterization of Methyl Glycosides.—Reducing powers were determined by the Nelson[17] method and provided an estimate of the amount of free sugars present. To test for the presence of easily hydrolyzed furanoside structure, approximately 5% solutions of the glycosides in 0.2 *N* sulfuric acid were heated for 1 hour at 70°,[18] after which reducing powers again were determined.

Methoxyl contents were determined by the method of Viebock and Schwappach.[19]

Periodate oxidation analyses were conducted according to the procedure described by Rankin and Jeanes.[20]

Optical rotations of aqueous solutions in 2-dm. tubes were measured at 4358 and 5461 Å. with a photoelectric polarimeter. The light source was an H-3 mercury lamp, and a Beckman spectrophotometer was used to isolate the desired wave length. Specific rotations were calculated from the anthrone values of glycoside concentration. The cuprammonium solution used for complex formation with the methyl glycosides was the "Cupra B" solution, prepared as described by Reeves.[5] Approximately 50 mg. of the glycosides were dissolved in 5 ml. of Cupra B, and the optical rotations at 4358 Å. were measured in 5-cm. cells by using the photoelectric polarimeter. The specific rotations at 4358 A. in aqueous solution were subtracted from the specific rotations in cuprammonium solution, and the differences were multiplied by the molecular weight of the glycoside, divided by its DP. The products are the molecular rotational shifts per glucoside unit shown in the last column of Table II.

Results and Discussion

Variation in yield of low molecular weight glycosides with time of methanolysis of NRRL B-512 dextran in 0.19 *N* methanolic HCl at reflux temperature is shown in Fig. 3. At 2, 3, 4, 6 and 8 hours methanolysis, 11, 15, 18, 25 and 34%, respectively, of the bonds in the dextran had been broken as determined by methoxyl analyses. Glycosides of low DP steadily increased in yield with increasing time of methanolysis as expected. After

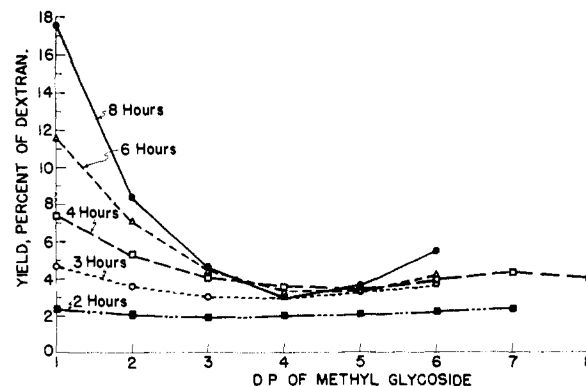

Fig. 3.—Yield of glycosides from NRRL B-512 dextran on methanolysis.

(17) N. Nelson, *J. Biol. Chem.*, **153**, 375 (1944).

(18) C. W. Wise, R. J. Dimler, H. A. Davis and C. E. Rist, *Anal. Chem.*, **27**, 33 (1955).

(19) E. P. Clark, *J. Assoc. Offic. Agr. Chemists*, **15**, 136 (1953).

(20) J. C. Rankin and A. Jeanes, THIS JOURNAL, **76**, 4435 (1954).

TABLE II

CHARACTERIZATION OF α-1,6'-LINKED METHYL GLYCOSIDES

Fraction	Methoxyl content, % Theoretical	Methoxyl content, % Found	Methyl glycoside, % of dry wt.	Reducing power as % glucose Before hydrol.	Reducing power as % glucose After hydrol.	R_f BMW[a] 4:2:3	R_f BPW[b] 1:1:1	$[\alpha]^{27}_{5461}$	$[\alpha]^{27}_{4358}$	Mol. rot. shift[c] in cuprammonium
1	16.0	15.7	98.0	0.07	0.35	0.53	0.57	62.0°	102.5°	28,800°
2	8.7	8.8	99.5	.13	.34	.42	.43	122.2	202.6	−18,400
3	6.0	6.0	98.5	.10	.33	.33	.32	153.8	254.3	−45,900
4	4.6	4.4	98.5	.20	.45	.25	.22	170.0	282.0	−59,300
5	3.7	3.4	99.0	.56	.70	.20	.16	182.6	303.3	−66,200
6	3.1	3.0	97.6	.41	1.12	.15	.10	197.5	327.0	−73,400
7	2.7	2.8	99.2	.59	0.83	.11		195.8	323.4	−75,200
8			98.2	.39	.52	.080		201.6	333.0	−77,300
9			98.4	.39	.75	.056		204.7	339.0	−79,800
10			97.0			.046		207.0	344.2	−80,000
11			96.5			.034		210.0	348.0	−81,500
α-Methyl glucopyranoside				0.71	0.87					
Dextran B-512E				.04	.24			243.1	389.0	−90,500

[a] Butanol-methyl cellosolve-water. [b] Butanol-pyridine-water. [c] Molecular rotational shift = M^{27}_{4358} (Cupra) − M^{27}_{4358} (water).

3 hours, however, production of glycosides of DP 4 to 8 was found to be 3 to 4%, regardless of heating time. This could be explained perhaps by the heterogeneity of the reflux mixture which would maintain a constant concentration of dissolved high molecular weight dextrans, so that rates of production and degradation of glycosides of intermediate size would attain a state of equilibrium. Time of methanolysis thus was not a critical factor in determining the yield of low DP glycosides, and for the large-scale preparation dextran was methanolyzed 6 hours.

From Fig. 1 it appears that good separation of the first 6 glycosides was achieved on the large cellulose column, using BMW (2:1:1) as the partitioning solvent, as evidenced by the fact that each fraction was followed by a volume of eluate containing comparatively little carbohydrate. Further evidence of the chromatographic purity of these fractions was provided by strip chromatograms of the middle portions of these fractions developed with BMW (4:2:3) and BPW (1:1:1). In no case was an impurity indicated. This also was true for the middle portion of fraction 7; however, chromatograms of higher fractions were streaked from the starting line and slight amounts of faster moving components were present in addition to the principal components in fractions 10 and 11. Recombination of the middle portions of fractions 6, 8 and 10 into one lot and fractions 7, 9 and 11 into another, followed by refractionation on smaller cellulose columns, removed the fast-moving components from fractions 10 and 11. Chromatograms of fraction 8 and above again were streaked from the starting line. This property was peculiar to the paper chromatograms, for a noticeable trailing edge did not appear in the elution of these fractions from the cellulose column; moreover, these fractions were eluted completely from the column by the developing solvent. An explanation for the failure of the high molecular weight glycosides to move completely from the starting line appears to be their tendency to crystallize from water solutions. Slow evaporation of water solutions of fractions 2 to 6 resulted in solids amorphous to X-rays, whereas fractions 8 to 11 crystallized and gave excellent X-ray diffraction patterns. Fraction 7 was intermediate in crystallinity. Both the rate and extent of solubility of the amorphous fractions would be expected to be greater than those of the crystalline fractions. Additional evidence that streaking and failure to move from the starting line was a solubility effect was given by chromatograms of fractions 8 and 9 deposited on the paper in 1 *N* NaOH solution. On development, these chromatograms showed single spots with no streaking or residue at the starting line.

Characterization of Fractions.—Methoxyl contents determined for fractions 1 to 7 are listed in Table II. Theoretical values for methyl glycosides having DP's corresponding to the fraction number also are listed; agreement of the two sets of values is within the experimental error of the methoxyl determination. Methoxyl analyses of fractions 8 to 11 were not performed, for it was considered that this method was too insensitive for differentiation of glycosides greater than DP 7. Column 2 of Table II lists values of the methyl glycoside content for each of the fractions determined by anthrone analysis of samples dried to constant weight. Although the deviation of these values from 100% for the first 9 fractions is little greater than expected from the precision of the anthrone method,[14] the consistently low values observed for all fractions indicates the presence of non-carbohydrate component.

All fractions were essentially free of reducing sugars as shown by results of reducing-power measurements listed in Table II. For comparison, values also are given for a sample of recrystallized α-methyl glucopyranoside and for undegraded dextran. Similarly, no easily hydrolyzed furanoside structures appear to have been introduced by methanolysis, as shown by the values in Table II for the reducing power determined after subjecting the fractions to mild acid-hydrolysis conditions. With the possible exception of fraction 6, increases in reducing power were small and about equal to those found for α-methyl glucopyranoside and undegraded dextran.

R_f-values determined from chromatograms of the fractions, developed either with BMW (4:2:3) or

BPW (1:1:1) are given in Table II, and form a series consistent with assignment of DP equal to the ordinal number of the fraction. This is demonstrated by the linear relationship between log $(1/R_f - 1)$ and *DP*, shown in Fig. 4, which is in accord with the prediction of Bate-Smith and Westall[21] for homologous series and with the observations by Jeanes, Wise and Dimler[22] on the hydrolysis products from the amylose fraction of starch.

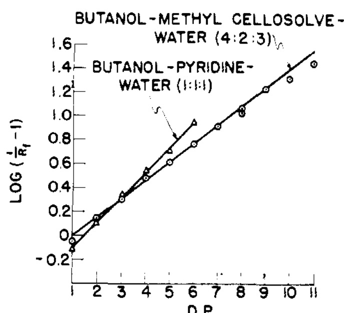

Fig. 4.—R_f-Values for methyl glycosides separated on cellulose column.

Elution of the fraction from the large cellulose column also obeyed this relationship which, in form appropriate to column chromatography, may be expressed

$$\log (V_n/V_s - 1) = a + bn$$

V_n is the total volume of eluate collected during the time required for elution of glycoside of $DP = n$, and V_s is the volume of eluate collected during the period required for the solvent to travel the length of the column. Constants a, b and V_s were computed from the volume of eluate at maximum concentrations of fractions 1, 2 and 3, given in column 4 of Table I. Excellent agreement was obtained between calculated (column 5, Table I) and observed values of V_n for fractions 4 to 7, which were eluted before the developing solvent was changed.

On periodate oxidation of methyl isomaltoside and its higher homologs, one molecule of formic acid should be liberated for each anhydroglucose unit in the chain. Results presented in Table III for 96 and 144 hours oxidation time show that yields of formic acid very close to theoretical were obtained for fractions 3 through 7. By comparison, formic acid yields for fractions 8 and 9 were slightly low, but were within the accuracy of formic acid measurements found by Rankin and Jeanes[20] for NRRL-B512 dextran. If no allowance were made for error in measurements, the results would permit as an upper limit about 10% singly-branched isomers in fractions 7 and 8. Other factors must have reduced the formic acid yield from fractions 1 and 2, for it seems likely that the presence of other glycosides would have been detected on the chromatograms in these cases. Periodate reduced was greater than that expected from the formic acid yield for fractions 1, 4, 7, 8 and 9. The excess, however, was no greater than that found in the case of B-512 dextran[20] based on its structure from methylation data.

(21) E. C. Bate-Smith and R. G. Westall, *Biochem. et Biophys. Acta*, **4**, 427 (1950).

(22) Allene Jeanes, C. S. Wise and R. J. Dimler, *Anal. Chem.*, **23**, 405 (1951).

Table III

Periodate Oxidation Analyses of α-1,6'-Linked Methyl Glycosides

	Reaction time, hours				
	72		96		144
Fraction	Periodate[a] reduced	Formic acid produced	Periodate reduced	Formic acid produced	Formic acid produced
1	1.97	0.951	2.01	0.968	0.972
2	3.91	1.91	3.91	1.95	1.95
3	6.06	2.94	5.95	2.99	2.99
4	7.93	3.91	8.22	4.01	3.99
5	10.4	4.90	9.98	5.00	4.96
6	12.0	5.90	12.0	5.98	6.04
7	14.2	6.91	14.2	7.03	7.00
8	15.7	7.77	16.5	7.90	7.86
9	17.9	8.83	18.6	8.94	8.86

[a] Moles per methyl glycoside.

Optical Rotation of Fractions in Aqueous and Cuprammonium Solutions.—Further evidence that the isolated products were consecutive members of a homologous series of oligosaccharide glycosides was provided by the interrelationship of the specific rotations of the fractions. Freudenberg[23] has shown that for a homologous series beginning with the disaccharide, a plot of the molecular rotation per polymer unit, $\bar{M}_n/n$, against $(n - 1)/n$ should be a straight line. As shown in Fig. 5, the data on optical rotation of the members of the glycoside series in water satisfy this relationship very well, except, as expected, for the monosaccharide derivative. The deviation of the point for B-512 dextran at $(n - 1)/n = 1$ from the linear relationship for the methyl isomaltoside series probably results from the branched structure of this polysaccharide.

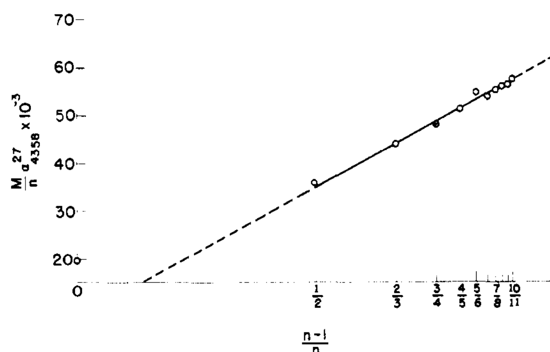

Fig. 5.—Freudenberg's relation for isomaltoside series.

It would be expected, of course, that methanolysis should produce a mixture of α- and β-anomers of each methyl glycoside. Comparison of the optical rotation for fraction 1 with that reported for α- and β-methyl glucosides[24] shows that this fraction contained 55% of the β-anomer. It may be assumed that the same ratio of anomers was present in each of the higher fractions. Conformance of the optical rotation values to the Freudenberg relationship provides strong support for this assumption, particularly for fractions of low *DP* in which anomeric configuration would have a considerable effect on the molecular rotation.

(23) K. Freudenberg, D. Friedrich and I. Bumann, *Ann.*, **494**, 41 (1932).

(24) F. J. Bates and associates, "Polarimetry, Saccharimetry and the Sugars," NBS Circular C-440, 1942.

Evidence on the nature of the copper complex formed and, therefore, of the glucopyranose ring conformation is provided by the difference in optical rotation in cuprammonium solution and in water.[5] The shift in molecular rotation for the glycosides of the isomaltose homologous series is shown in Table II. Since all of the units in each of the glycosides have hydroxyls on carbon atom 2, 3 and 4 available for complex formation, one might expect the shift in rotation per polymer unit to be essentially constant and nearly equal to that of the monomeric glycoside, methyl D-glucopyranoside. This assumes, however, that the terminal units of the polymer chain make the same contributions to the rotational shift as interior anhydroglucose units. The progressive change in rotational shift to more negative values indicates that one or both of the end units reacted differently from the others in terms of copper complex formation.

Theoretically, the rotational shift for the members of a homologous series should obey the same type of relationship as the molecular rotations, that is

$$\frac{S_n}{n} = S' + S'' - \frac{S_\infty}{\infty} + \frac{2(n-1)}{n}\left[\frac{S_\infty}{\infty} - \frac{S' + S''}{2}\right]$$

where Sn/n is the molecular shift per unit of the polymer of $DP = n$, S' and S'', the respective shifts for the two terminal units, and S_∞/∞ the molecular rotational shift per unit of the infinitely large polymer. The test of this relationship for the isolated fractions is shown in Fig. 6, in which the molecular rotational shift per polymer unit is plotted against $(n - 1)/n$. The expected linear relationship exists up to $n = 6$ or 7. For fractions above DP 7 deviations from linearity in the same direction shown by the undegraded dextran $(n - 1)/n = 1$ are apparent, possibly as a result of the presence of some branched polymers.

The data do not permit separate evaluation of S' and S'', respective contributions of the two terminal units to the rotational shift. The methyl glucoside end, however, bears substituents on both number 1 and number 6 carbon atoms, and in this respect resembles interior units of the polymer more closely than does the opposite end of the chain. Assuming the methyl glycoside end to have the same rotational shifts as interior units, the contribution of the other terminal unit with the 6-hydroxyl unsubstituted can be determined by extrapolation to be 61,000°. Molecular rotational shifts of several dextrans are consistent with this value for the contribution of the non-reducing end groups and thus support the above assumption.

The value of S_∞/∞, the shift for an α-1,6'-linked anhydroglucopyranose unit, was obtained from Fig. 6 by extrapolation to $(n - 1)/n = 1$, giving a value of −99,000°. This is about half of the shift observed by Reeves[5] for methyl 4-methyl D-glucopyranoside, which is capable of forming a copper complex only at the 2,3-hydroxyl positions. These observations, then, strongly suggest that the presence of a glucosyl unit on the number 6 hydroxyl leads to a marked preference for complex formation at the 2,3-position compared with the 3,4-position, as has been suggested also by Reeves. Such a behavior could be a result of the presence of the B2 asymmetric *cis* ring conformation[5] in which complex formation is possible at the 2,3-position but not at the 3,4-position. This conformation appears unlikely, however, because of steric obstructions as shown by measurements on models,[25] and it seems more probable that small deviations from the preferred symmetrical *trans* form C1 exist in an α-1,6'-linked glucosan which favor complexing at the 2,3-position.

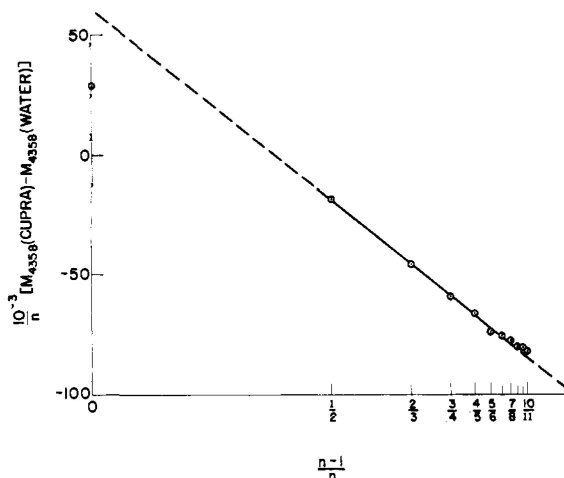

Fig. 6.—Molecular rotational shift on copper complex formation for methyl isomaltoside series.

Regardless of the reason for the rotational shift of −99,000° for the anhydroglucose unit of the infinite 1,6'-linked linear polymer, this information on the apparent effect of polymer structure on the copper complex formation will be of value in the application of rotation measurements in cuprammonium to studies of the structure of different dextrans. The large negative rotational shift calculated for anhydroglucose units having a glucosidic linkage on carbon number 6 provides an explanation for the negative rotation of B-512 dextran in cuprammonium. Further application of these observations to B-512 and other dextrans will appear in a forthcoming publication.

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