Reactions of Oligosaccharides. II. Dextrose Equivalents[1]

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ABSTRACT

The Corn Industries Research Foundation's modification of the Lane and Eynon procedure for determining reducing sugars in corn syrup was used to obtain the dextrose equivalents (DE's) for a series of D-glucose polymers ranging from the di- to the hexasaccharide. Oligosaccharides were obtained by macro paper chromatography of corn starch hydrolysates. Data obtained were calculated in several ways, to compare reducing powers of the members of the series. Application of values obtained to various corn syrup compositions revealed a remarkably close agreement in observed and calculated DE's. Preliminary experiments were made to determine the composition of residual carbohydrates and of end products in order to elucidate the extent and course of oxidation reactions.

In the first paper of this series (1), reducing ability of oligosaccharides prepared by macro paper chromatography was described in terms of ferricyanide reduction. Conditions were sought under which reaction would be stoichiometric, and some progress toward this goal was made. For the foreseeable future, however, reliance on empirical methods will still be necessary.

The term commonly used in the corn wet-milling industry to express relative reducing power of starch hydrolysates is "dextrose equivalent" (DE). According to procedure E-26 in the Standard Analytical Methods Handbook of member companies of the Corn Industries Research Foundation (2), DE is defined as "reducing sugars expressed as dextrose and calculated as a percentage of the dry substance." Procedure E-26 is a modification of the Lane and Eynon titration method (3) in which the reducing sugars contained in corn syrup are oxidized by Fehling's solution. As in most reducing-sugar oxidations, the method is subject to criticism because the extent of oxidation depends on reagent concentration, time, temperature, pH, and other variables. To circumvent this, the general practice followed in determining reducing sugars has been to establish a set of rigid conditions for a particular method. In the Lane and Eynon method, in which this has been done, the reproducibility of the results depends on the degree to which the conditions are adhered to (4–8).

The dextrose equivalent concept assumes that the reducing sugars in corn syrup behave like dextrose. Theoretically, the DE of maltose should be 52.6 (the molecular weight of dextrose divided by the molecular weight of maltose times 100). Actually, values ranging from 54 to 62 have been reported. Whether this deviation is due to differences in the reaction conditions or to inherent differences in the stoichiometry of the reaction has not been established. The homologous series of D-glucose polymers higher than maltose normally found in corn syrup is an interesting set of compounds for studying reactions of this type. In this work the standard DE procedure was run on the individual D-glucose polymers of degree of polymerization 2 through 6 (DP_2 through DP_6), to determine the effect of increased chain length on deviation from the theoretical DE.

MATERIALS AND METHODS

Oligosaccharides

The D-glucose polymers of DP_3 through DP_6 were separated from corn starch

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hydrolysates by macro paper chromatography (9) and isolated as amorphous powders by freeze-drying aqueous solutions of the eluted sugars. The solvent employed in the separation was composed of 1-propyl alcohol, ethyl acetate, and water in the ratio of 7:1:3 by volume. The polymers were rechromatographed twice and showed only one spot on a chromatogram. As a check on the purity of the polymers they also were run with a solvent composed of ethyl acetate, pyridine, and water in a ratio of 8:2:1 by volume, and again only one spot was observed.

The lyophilized powders were further dried in an Abderhalden apparatus operated at high vacuum with phosphorus pentoxide used as a desiccant and 1-propanol (b.p. 97°C.) in the reflux chamber.

Recrystallized maltose hydrate was chromatographed to remove traces of higher sugars, and dried in a similar manner to obtain the anhydrous sugar.

Other Reagents

Analytical-grade reagents were used unless otherwise mentioned.

DE Procedure

Fehling's solution was prepared by mixing equal portions of solution A containing 34.64 g. of copper sulfate pentahydrate per 500 ml., and solution B containing 173 g. of Mallinckrodt NF Rochelle salt and 50 g. sodium hydroxide per 500 ml. The copper reagent concentration was adjusted by addition of copper sulfate or water so that 25.0 ml. of reagent oxidized exactly 12.02 ml. of standard 1.0% dextrose solution made up from redried National Bureau of Standards anhydrous dextrose. Aqueous D-glucose polymer solutions were prepared with sufficient anhydrous sugar to give a concentration in percent solids equal numerically to the degree of polymerization, so that the titration volume would be about 12 ml.

TABLE I.   DEXTROSE EQUIVALENTS OF D-GLUCOSE POLYMERS[a]

	Theoretical	Observed
Monosaccharide	100	100
Disaccharide	52.6	58.0
Trisaccharide	35.7	39.5
Tetrasaccharide	27.0	29.8
Pentasaccharide	21.7	24.2
Hexasaccharide	18.2	20.8

[a]Determined by the CIRF modification of the Lane and Eynon procedure.

TABLE II.   STOICHIOMETRY OF THE COPPER-SUGAR REACTION

Polymer	Polymer meq.	meq.Cu/ meq. Polymer	meq. Polymer/ meq. Dextrose
Monosaccharide	0.67	5.2	1.00
Disaccharide	0.61	5.7	0.91
Trisaccharide	0.61	5.7	0.91
Tetrasaccharide	0.60	5.8	0.90
Pentasaccharide	0.60	5.8	0.90
Hexasaccharide	0.59	5.9	0.88

Cu in 25 ml. of reagent, meq. = 3.47.

Meq. of Polymer = (titration vol.) $\times$ (conc.) $\times$ 1,000/mol. wt.

In the Lane and Eynon procedure, 25.0 ml. of Fehling's solution heated to the boiling point over an open flame is titrated with the sample solution to within 0.5 ml. of the end point. Then after 2 min. of boiling, 2 drops of 1% methylene blue indicator is added and the titration is rapidly continued to the disappearance of the blue color. A trial titration is run first to determine the approximate end point.

RESULTS AND DISCUSSION

The values obtained for the DE's of the oligosaccharides are shown in Table I, together with the theoretical DE's one would obtain if the terminal reducing unit reacted in the same manner as it does in dextrose. From these values it is seen that the actual DE's are about 10% higher than expected. In Table II the data are recalculated in terms of meq. of polymer oxidized. Here it is seen that for the disaccharide and the higher polymers about 0.60 meq. of sugar reacts with 25.0 ml. of copper reagent as compared to 0.65 meq. of dextrose.

Whether the observed differences can be correlated exactly with specific reactions is open to speculation; however, there are many areas in which knowledge of the DE's of the individual oligosaccharides is of immediate value.

Using the CIRF tables of composition of various corn syrups (10), one can calculate the total DE based on the DE contribution of each component (Table III).

TABLE III. ACID-CONVERTED CORN SYRUPS

DP of Saccharide	30 DE[a] %	Composition 40 DE %	50 DE %	30 DE	D.E. Contribution 40 DE	50 DE
1	10.4	16.9	25.8	10.4	16.9	25.8
2	9.3	13.2	16.6	5.4	7.7	9.7
3	8.6	11.2	12.9	3.4	4.4	5.1
4	8.2	9.7	10.0	2.4	2.9	3.0
5	7.2	8.3	7.9	1.7	2.0	1.9
6	6.0	6.7	5.9	1.2	1.4	1.2
7+b	50.3	34.0	20.8	6.8	4.6	2.8
Calculated DE				31.3	39.9	49.5

[a]Percent composition taken from CIRF Critical Data Tables believed to be ±1% accurate.

[b]Calculated DE of the hepta+ polymers is 13.6 based on average DP of 9 for higher sugars.

For the 30 DE syrup this calculated value is 31.3. The discrepancy is probably a result of estimating that the average degree of polymerization of the DP_7^+ polymers is about 9. In the other syrups the calculated values check to within the accuracy of the method. In Table IV are shown the calculated and actual DE's of syrups made up by blending various amounts of D-glucose polymers. The syrups were picked to match types of commercial high-DE products made by three different processes: a syrup of high-DE acid-converted type, an acid-enzyme type, and an acid-dual-enzyme type. In all three cases the agreement between observed and calculated values is within the limits of accuracy of the method.

Other uses are possible, such as calculation of the extent to which a corn syrup should be converted to obtain a product of specific DE and composition, or the blending of various syrups to obtain a specific DE and composition.

In calculating the DE contribution of the sugars higher than the hexasaccharide, the relationship $(12.02 \times 100)/$ (polymer mol. wt. $\times$ 0.6 meq.) = DE was used. This

TABLE IV. SYNTHETIC HIGH D.E. SYRUP

	Acid-converted type			Acid-enzyme type	
DP of Sac-charide	Compo-sition %	DE Contri-bution	DP of Sac-charide	Compo-sition %	DE Contri-bution
1	36.2	36.2	1	37.4	37.4
2	19.5	11.3	2	31.0	18.0
3	13.2	5.2	3	10.1	4.0
4	8.7	2.6	4	3.1	0.9
5	6.3	1.5	5	3.5	0.8
6	4.4	0.9	6	2.6	0.5
7	4.7	0.8	7	4.8	0.8
8	2.4	0.4	8	2.4	0.4
9	4.7	0.1	9	4.8	0.1
Calculated DE		59.2	Calculated DE		62.9
Observed DE		60.0	Observed DE		63.7

	Acid-dual-enzyme type	
DP of Sac-charide	Compo-sition %	DE Contri-bution
1	43.0	43.0
2	40.0	23.5
3	2.0	0.8
4	2.0	0.6
5	1.0	0.2
6	5.0	1.1
7	2.8	0.5
8	1.4	0.2
9	2.8	.....
Calculated DE		69.9
Observed DE		70.2

is based on the assumption that the sugars above DP_6 behave the same as the series DP_2 to DP_6.

Preliminary experiments were carried out on residues collected from oxidation of the polymers to determine the extent of degradation on D-glucose units beyond the terminal reducing unit. Acid hydrolysis failed to yield precise amounts of D-glucose. Currently, enzymatic studies and chromatographic separations are in progress to further elucidate the nature of the reaction products.

Acknowledgments

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