whereupon the cooled solution was poured on ice (25 g.) and the separated solid removed by filtration and purified by crystallization from ethanol; m. p. 193–195° (dec.).

Anal. Calcd. for $C_{20}H_{26}O_{14}$: C, 48.98; H, 5.34. Found: C, 48.76; H, 5.31.

We acknowledge the assistance rendered in a portion of this work by Messrs. Ralph S. Klopper and Stephen Olin.

Summary

1. The known *keto-d*-fructose pentaacetate has been synthesized by the reaction between acetic acid and 1-diazo-1-desoxy-*keto-d*-fructose tetraacetate.

2. Acetic acid was reacted with 1-diazo-1-desoxy-*keto-d*-glucoheptulose pentaacetate (I) to produce *keto-d*-glucoheptulose hexaacetate (II), also obtainable by the acetylation of 1-bromo-*keto-d*-glucoheptulose pentaacetate.

3. Mucyl dichloride tetraacetate (III) was treated with diazomethane to produce the bisdiazomethyl ketone (IV) from which the 1,8-dichloride and the 1,8-diacetoxy (V) derivatives were formed. The latter is an acetate of a diketose, a new type of structure in the sugar field.

4. The above reactions establish a new synthesis of *keto*-acetates from aldose derivatives of lower (one or two carbon atoms) carbon content.

COLUMBUS, OHIO RECEIVED JULY 3, 1942

[CONTRIBUTION FROM DEPARTMENT OF CHEMISTRY, IOWA STATE COLLEGE]

Structure of the Dextrins Isolated from Corn Sirup[1]

BY MELVIN LEVINE, JOSEPH F. FOSTER AND R. M. HIXON

The structural differences between the different starches and starch fractions, especially with regard to the questions of branching and non-reducing fractions, would appear to be somewhat clarified by a more complete study of the structure of the low molecular weight products of hydrolysis. In the older literature Brown[2] concluded that these products consist essentially of maltose and a "stable dextrin" of definite molecular size (about 40 glucose units) with a specific rotation of about 196° and a reducing power equivalent to 5.5% maltose, or about the value which would be expected for a molecule of this size if it terminated in a reducing glucose molecule. These results have been partially accepted in the corn sirup industry where "dextrin" is considered to be a definite substance having the rotation reported by Brown, but considered to be non-reducing.[3] From a theoretical standpoint these degradation products would be expected to consist of a mixture of glucose polymers of varying chain length, and with reducing power and specific rotation[4] depending on the chain length. If the original starch contains branching, as the present evidence indicates, these molecules might be expected to be further complicated.

The method which we are reporting in this paper for isolating the dextrins from corn sirup was developed primarily to make these materials available in relatively large quantities for physiological investigations. The availability of such dextrins together with the theoretical importance of a more complete knowledge of their structure has encouraged a rather detailed investigation of these materials, especially with regard to the questions of branching and the presence of any non-reducing fractions.

Experimental

Isolation of Crude Dextrins from Corn Sirup.—The conditions used for the isolation of the dextrins were selected after a study of the solubilities of glucose and maltose in aqueous alcohol (Fig. 1). Fifteen pounds of corn sirup[5] was weighed into a five-gallon container. Enough absolute methyl alcohol to bring the concentration to 80–85% was added, the mixture heated to 55° in a water-bath and stirred thoroughly until a homogeneous mixture was obtained, which was allowed to stand at approximately 45° until the supernatant alcoholic extract was clear (twenty-four to forty-eight hours) and was then decanted. The residual heavy sirup was again extracted with approximately three volumes of 80% alcohol by stirring at 55°, allowing to settle at 40°, and decanting the clear supernatant liquor. This extraction process was repeated four times.

(1) Journal Paper No. J-1007 of the Iowa Agricultural Experiment Station, Ames, Iowa; Projects No. 688 and 516. Supported in part by a grant from the Corn Industries Research Foundation.

(2) Brown and Millar, *J. Chem. Soc.*, **75**, 315 (1899).

(3) For one method of analysis and a review of the general methods in use see Fetzer, Evans and Longnecker, *Ind. Eng. Chem., Anal. Ed.*, **5**, 81 (1933).

(4) Freudenberg, Friedrich and Bumann, *Ann.*, **494**, 41 (1932).

(5) The corn sirup used was Amaizo Crystal White, 41 purity, 43° Bé., furnished by the American Maize Products Company, Roby, Ind.

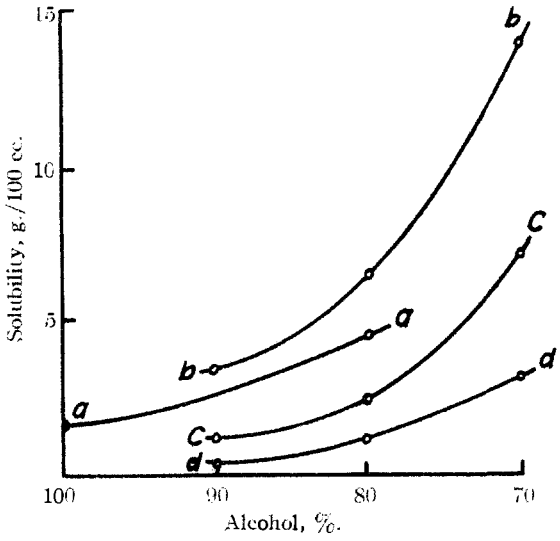


Fig. 1.—Solubility of maltose and glucose in aqueous alcohol: a, glucose in C_2H_5OH at 20°; b, maltose in CH_3OH at 40°; c, maltose in CH_3OH at 25°; d, maltose C_2H_5OH at 25°. The data for glucose are from Hudson and Yanovsky, THIS JOURNAL, **39**, 1013 (1917). The data for maltose were determined in this Laboratory by averaging the values calculated from rotation and reducing value.

The heavy, sticky residue was thinned somewhat with water, heated to 60° and filtered through a cotton filter cloth with the aid of suction. Absolute methanol was added to the filtrate to bring the concentration up to 85%, the sirup allowed to settle, then dehydrated and pulverized with absolute methanol. The powdered dextrin was recovered by filtering with the aid of suction and dried in a vacuum oven at 45° for forty-eight hours. The final products were slightly hygroscopic white powders having a slightly sweet taste, specific rotation of about 175°, and reducing value (R_{Cu}) by the alkaline ferricyanide method[6] of about 750 to 850 (maltose = 1900).

(6) Farley and Hixon, *Ind. Eng. Chem., Anal. Ed.*, **13**, 616 (1941).

Fractionation of Dextrins.—A weighed amount of crude dextrin was dissolved in twice its weight of distilled water, filtered, precipitated by the addition of four volumes of absolute methanol, allowed to stand till clear and the supernatant liquid poured off. The soluble fraction was recovered by evaporating under reduced pressure, and both soluble and insoluble fractions dried. Reducing power and rotation were run on the fractions, after which they were subjected to further fractionation by the same method. A typical fractionation is shown graphically in Fig. 2. The fractions used in the chemical studies discussed below were prepared by this method of fractionation.

To determine the completeness of removal of maltose in this method of fractionation, a mixture consisting of 3 g. of maltose and 10 g. of dextrin V (mol. wt. 1800 by iodine titration) was subjected to a single fractionation in 80% methanol. The insoluble fraction consisted of 7.2 g., mol. wt. 1950; the soluble fraction 4.5 g., mol. wt. 530. If the latter fraction be assumed to contain all of the maltose plus 1.5 g. of the dextrin, the mean molecular weight would be about 490.

Acetylation of Dextrins and Fractionation of the Acetates.—Satisfactory acetylation could be attained by any of the variations of the pyridine–acetic anhydride method.[7,8,9] A 100-g. sample of a dextrin having a reducing value of 850 was dissolved in 400 cc. of pyridine and allowed to stand for forty-eight hours. Then 500 cc. of pyridine and 500 cc. of acetic anhydride were added and the mixture shaken. After standing for forty-eight hours the solution was poured into about 10 liters of water, the precipitate collected on a filter and washed well with about 5 liters of water; yield 220 g. of white powder.

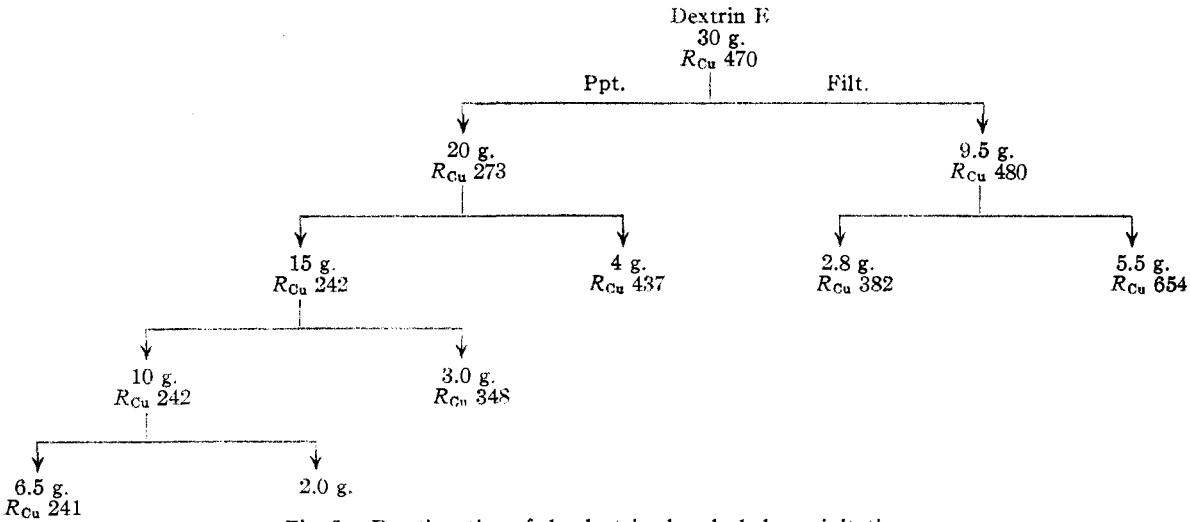


Fig. 2.—Fractionation of the dextrins by alcohol precipitation.

This material was repeatedly fractionated by dissolving in boiling absolute methanol and then cooling the solution in an ice-bath for about three hours until the dextrin acetate had settled. This came down as a white, very heavy

(7) Behrend and Roth, *Ann.*, **331**, 359 (1904).
(8) Haworth, Hirst and Plant, *J. Chem. Soc.*, 1214 (1935).
(9) Higginbottom and Richardson, *J. Soc. Chem. Ind.*, **57**, 234 (1938).

sirup which hardened on standing and was broken up and pulverized with a glass rod. The solution was filtered, the filtrate evaporated to dryness, and both the soluble and insoluble fractions dried and pulverized. Results of this type of fractionation are given in Fig. 3.

Oxidation of Dextrins and Isolation of the Dextrinic Acids.—It was found that these dextrins could be converted quantitatively to the potassium salts of the dextrinic acids by a large-scale modification of the Kline and Acree method for determining aldose sugars.[10] A small sample of the dextrin was first titrated with iodine and alkali according to their directions to determine the exact reducing value. Then a sample of from 10 to 20 g. was dissolved in a small volume of water, placed in a three-neck reaction flask equipped with a mechanical stirrer and two dropping funnels, and the calculated amount of iodine and potassium hydroxide (each approximately 0.1 *N*) added, the caustic being permitted to drop continuously and the iodine being added in about 5-ml. portions as the solution became colorless. The addition required two or three hours and an additional three hours with stirring was allowed for completion of the reaction. The solution was concentrated to a thick sirup under reduced pressure, and the oxidized dextrin isolated by repeatedly adding methyl alcohol to a concentration of 85%, centrifuging out the solid, which at this point was rather gummy, taking up in water and repeating the alcoholic precipitation until the aqueous solution gave no test for iodide. The solid was then shaken up several times with ether, and finally ground in a mortar and dried, giving a perfectly white powder. Yields were usually 90 to 95% of the theoretical.

The potassium salts were analyzed for potassium by ashing 20-30-mg. samples with a drop of concentrated sulfuric acid in a small electric muffle, and weighing the sulfate formed. The chain length calculated from per cent. of potassium was found to agree very well with that calculated from the amount of iodine and caustic used in the oxidation. For example, one fraction having a chain length of 14.2 glucose units by iodine titration was oxidized and the derivative found to contain 1.67% potassium, from which the chain length was calculated to be 14.4 glucose units.

Maltose was also oxidized to potassium maltobionate by the above method, but the derivative was found to be very hygroscopic and much harder to work with than in the case of the larger molecules.

Methylation of Dextrins.—Attempts to methylate these dextrins by the various methods which have been used for carbohydrates showed that by far the best results could be obtained by means of sodium and methyl iodide in liquid ammonia, which was first applied to starch by Freudenberg and Boppel.[11] By this method the theoretical per cent. of methoxyl could be attained in one treatment, and recovery was practically quantitative. However, due to the greater solubility of these materials in both water and ammonia, it was found impracticable to change solvent during the methylation, and recovery of the methylated product had to be effected by evaporating off the ammonia, and exhaustively extracting the thoroughly dried and pulverized residue in a Soxhlet extractor with chloroform, instead of adding the residue to boiling water as was done by these workers.

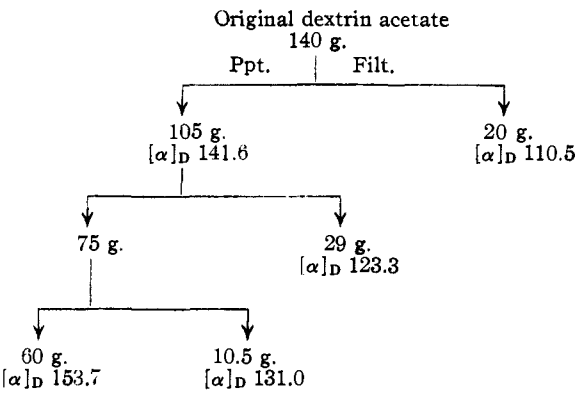


Fig. 3.—Fractionation of dextrin acetates.

Ten grams of a dextrin having an average chain length calculated from iodine titration of eleven glucose units was methylated by this procedure: yield, 12.5 g. of cream colored powder or 99% of theoretical (45.5% methoxyl, calculated for an 11 glucose dextrin, 46.3). Five grams of this material was hydrolyzed and the di-, tri- and tetramethyl glucose separated by the method of Bell,[12] which involves partition of tri- and tetramethyl glucose between chloroform and water. In this way, 0.515 g. of crystalline tetramethyl glucose was isolated (52.3% methoxyl, calculated 52.5). This corresponds to a chain length of 10.6 glucose units assuming no tetramethyl glucose to be lost. The amount of dimethyl glucose obtained was very small (about 30 mg.).

To check this result 3.8 g. of the methylated dextrin was hydrolyzed, converted to the glucosides, and analyzed for tetramethyl glucose by the method of fractional distillation.[13] By this method, 0.424 g. of tetramethyl methylglucoside was found in the distillate, and the chain length calculated to be 10.3 glucose units.

The molecular weight of the methylated dextrin was determined by two physical methods. By viscosity in chloroform, using Staudinger's equation and his value of K_m (namely, 1×10^{-4}),[14] the value obtained was 9700; cryoscopically, using bornyl bromide as solvent, 1530. This last value is in reasonable agreement with the value calculated from the chain length, or about 2000.

The salts of the dextrinic acids have also been methylated completely and in good yields following the same procedure as with the dextrins. To date no study of the methylated derivatives has been carried out.

Reaction of the Dextrins with Phenylhydrazine.—The procedure first tried was essentially that used by Bergmann and Machemer with the acetylated cellulose dextrins,[15] which involves preparation of the derivative in liquid phenylhydrazine, isolation by pouring into ether, and purification by solution in 50% acetic acid and precipitation with absolute methanol. Much better results were obtained in the case of the unacetylated materials by

(10) Kline and Acree, *Ind. Eng. Chem., Anal. Ed.*, **2**, 413 (1930).
(11) Freudenberg and Boppel, *Ber.*, **71**, 2505 (1938).
(12) Bell, *Biochem. J.*, **29**, 2031 (1935).
(13) Haworth and Machemer, *J. Chem. Soc.*, 2270 (1932).
(14) Staudinger and Eilers, *ibid.*, 2270 (1932).
(15) Bergmann and Machemer, *Ber.*, **63**, 322 (1930).

eliminating the use of acetic acid and using benzene instead of ether as the precipitant. The procedure finally established was as follows. One gram of the dextrin was dissolved in 5 cc. of phenylhydrazine by heating under reflux at 130° for two hours. The reaction mixture, after cooling, was poured with stirring into 50 cc. of benzene. The precipitate was recovered by filtration, washed several times with ether, thoroughly dried, pulverized, and extracted with ether in a Soxhlet extractor for twenty-four hours. By this procedure the recovery of the derivative was more complete and the results more consistent. The derivatives were analyzed for nitrogen by the micro Dumas method, and the molecular weight of the carbohydrate residue calculated.

These derivatives are amorphous powders, characteristically yellow in color in contrast to true phenylhydrazones, and are extremely susceptible to hydrolysis. When dissolved in water they give off a very noticeable odor of phenylhydrazine, and ether extraction of the aqueous solution or boiling with activated charcoal results in the removal of from 25 to as high as 85% of the nitrogen. For this reason it was at first thought that the materials were simply adsorption complexes; however, the fairly close agreement in the case of the smaller fractions between the molecular weight from nitrogen and iodine titration indicated that a definite reaction did take place.

To further investigate this question the derivatives of glucose and maltose were prepared following exactly the procedure used for the dextrins. The maltose derivative had the same characteristic yellow color as the dextrin derivatives and had the same amorphous appearance under the microscope. The glucose derivative was somewhat more orange in color and was essentially amorphous although there did seem to be some small crystals present which were birefringent. The rotations could not be determined accurately due to the dark color of the aqueous solutions, but the values were about −40° for the glucose derivative and +70° for the maltose derivative. Both derivatives melted over a wide range, indicating them to be non-homogeneous (maltose derivative, 115–118°; glucose derivative, 111–120°). Nevertheless the nitrogen analysis was in both cases very close to the theoretical (maltose derivative 6.46%, calculated 6.48; glucose derivative, 10.2, calculated 10.4). The maltose derivative showed the same instability in water as the dextrin derivatives, at least 25% of the phenylhydrazine being extractable. It is interesting to note that if the aqueous solution is exhaustively extracted with ether and then allowed to stand a few days, more phenylhydrazine can be removed, indicating that a reversible equilibrium is involved. The glucose derivative gave an odor of phenylhydrazine when dissolved, but the amount extractable was negligible.

The phenylhydrazine derivative of an acetylated dextrin was also prepared, following essentially the procedure of Bergmann and Machemer.[15] It was found that part of the acetyl groups were removed by the phenylhydrazine so that the derivative had to be reacetylated. The derivative was found to contain 1.70% nitrogen and 43.2% acetyl, from which the molecular weight of the carbohydrate residue was calculated to be 862, in good agreement with the value calculated from iodine titration, namely, 850.

Specific Rotations of the Dextrins.—Before running specific rotations the dextrins were carefully dried in a vacuum oven at 60° for twenty-four hours. Rotations were run at 25° on 1% solutions in water, using sodium D light. In Table I these results are compared with the values calculated assuming the additivity of molecular rotations as has been done by Freudenberg with the smaller molecules.[4] A value of 46,000 was assigned for the molecular rotation of maltose (or the sum of the reducing and non-reducing terminal glucoses in the dextrins) and 32,400 for the intermediate glucoses, the value for maltose being obtained from the observed rotation and iodine molecular weight and the value for the intermediate units by assuming a rotation of 200° for an infinitely long chain. The equation used is thus

$$\alpha_M = 46000 + \frac{M - 348}{162}(32400)$$

where α_M is the molecular rotation and M the molecular weight. The specific rotation is of course obtained by dividing the molecular rotation by the molecular weight.

TABLE I

COMPARISON OF SPECIFIC ROTATION CALCULATED FROM IODINE AND R_{Cu} REDUCING VALUES WITH THE OBSERVED VALUES

Fraction	Molecular wt. I_2	Weight from R_{Cu}	$[\alpha]_D$ calcd. from I_2	$[\alpha]_D$ observed	$[\alpha]_D$ calcd. from R_{Cu}
Maltose	348	342	...	132	...
A	310	354	118	114	148
B	469	461	150	149	149
C	888	734	172	172	168
M	924	778	175	175	170
F	1422	1036	184	184	178
V	1795	1383	187	187	183
R	1778	1340	187	185	183
E	1902	1450	188	185	183

Discussion

As can be seen from Fig. 2 the results of alcohol fractionation are not very satisfactory. The recovery is poor, the fractionation is slow and the materials very hard to work with. Nevertheless, from the crude dextrin, fractions were obtained having mean molecular weights ranging from less than the value of maltose up to about 26 glucose units, as calculated from iodine reducing value. The fractions are obviously heterogeneous since in no case was a fraction obtained which could not be further fractionated, although, in most cases, by the time the molecular weight had reached a value corresponding to about 25 glucose units the amount of the fraction was too small to permit further treatment. The result of the fractionation of the known mixture of maltose and dextrin V indicates that the higher fractions could not contain any appreciable amount of maltose or glucose, and it seems probable that the distribution of molecular sizes is fairly narrow.

Haworth and associates[16] have investigated the fractionation of the dextrins through their acetates with satisfactory results. The results obtained here indicate a fairly sharp fractionation as evidenced by the large difference in rotation between the fractions. The advantage of more rapid fractionation is, however, offset by the necessity of acetylating and regenerating the dextrins.

The excellent check between the per cent. potassium in the dextrinic acids and the iodine reducing value of the original dextrins indicates that the reaction is, as formulated by Kline and Acree,[10] a simple oxidation of aldehyde groups to the carboxylic acids, and that no iodine is used otherwise (for example, adsorbed). From this it would seem that iodine titration is the best criterion of chain length in these low molecular weight dextrins. In Fig. 4 the molecular weights calculated from R_{Cu} are compared with the corresponding values calculated from iodine reducing power. Results obtained in this Laboratory with higher molecular weight fractions indicate that the R_{Cu} molecular weights are too low by an almost constant value and hence it would appear that the curve bends upward and becomes parallel with the theoretical as is also indicated in the figure. It should be mentioned also that iodine methods are not very reliable in the case of very high molecular weight materials where tenacious adsorption occurs.

The reliability of the iodine values in this range is further indicated by the agreement between the specific rotations and the values calculated from the apparent molecular weights (Table I). Low observed values can be explained by moisture. In several cases the observed values are below those calculated from iodine molecular weight, but above those calculated from R_{Cu} molecular weight. Obviously specific rotation is not a very sensitive function for a high molecular weight fraction; nevertheless, even in the highest fractions studied here the calculated increase of specific rotation is approximately 1.5° per glucose unit.

The close agreement between the chain length calculated from reducing value and tetramethyl glucose analysis, even when we consider the limited accuracy of the available methods for separation of the methyl glucoses (the maximum relative accuracy is probably not more than about 5–10%) indicates the chains to be essentially linear. The almost complete lack of dimethyl glucose, which would arise at the points of branching, supports this conclusion (30 mg. of dimethyl glucose would permit a maximum of one branch to about 160 glucose units, or about 6% of branched molecules). This result is to be expected for fractions of this size from simple probability considerations, if we assume the 1–4 and 1–6 linkages to be broken at the same rate. It should perhaps be pointed out that the method of tetramethyl assay is not a very sensitive criterion for ascertaining whether or not a small portion of the molecules are branched. For example, a straight-chained fraction of ten glucose units would give 10% of tetramethyl glucose, whereas if even 25% of the molecules were branched only about 12.5% of tetramethyl glucose would be obtained. Hence the absence of dimethyl glucose is, perhaps, a safer criterion.

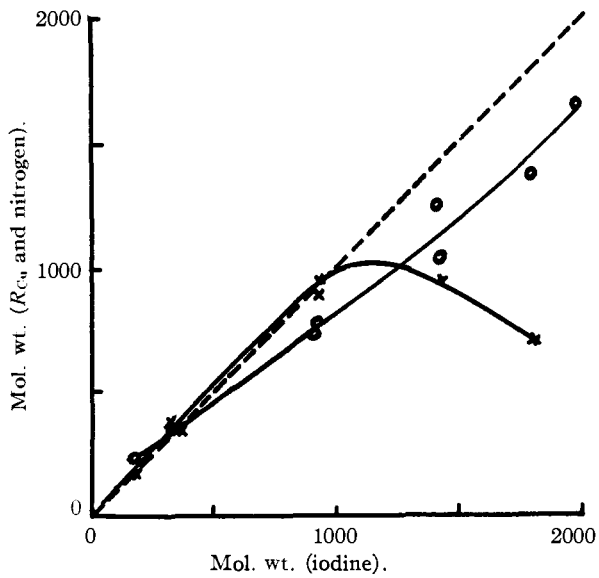
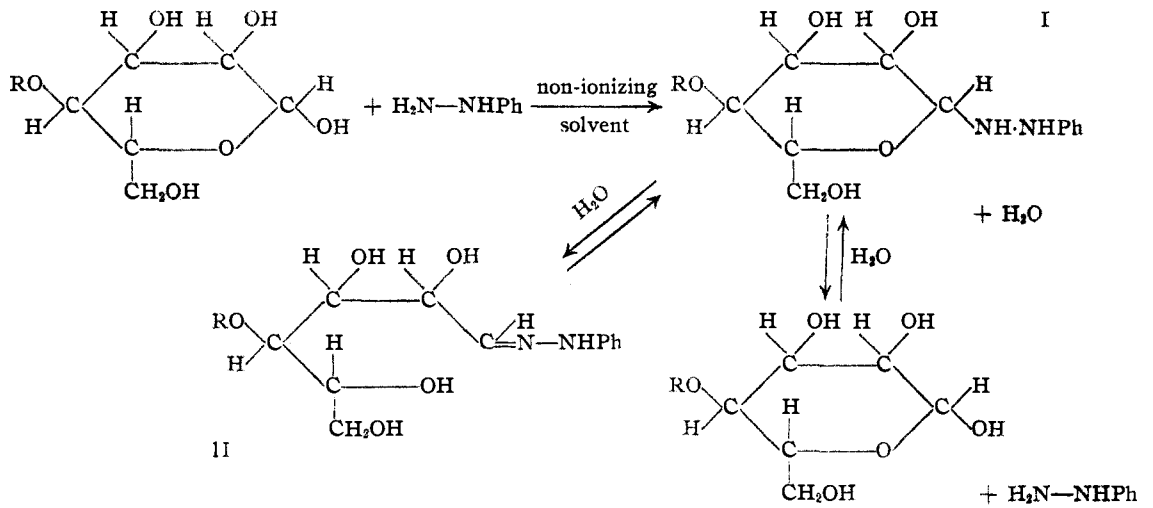


Fig. 4.—Comparison of molecular weights calculated from R_{Cu} and per cent. nitrogen in the phenylhydrazine derivatives, with the values obtained from iodine reducing values. Molecular weights were calculated from R_{Cu} by assuming the value for maltose (1900) to be correct and using the equation $M = 342 \times 1900/R_{Cu}$. The crosses are nitrogen values; the circles R_{Cu} values.

In considering the possible presence of non-reducing dextrins, two distinct types must be considered. In the first place, there is the possibility of dextrin structures in which the terminal aldehyde group is bound in a non-reducing fashion but the non-reducing end is free. This type of molecule would be detected by the tetramethyl end-group analysis since it would lead to a preponderance of tetramethyl groups over reducing groups. Conclusions based on this evidence are, of course, subject to the same experimental inac-

(16) Haworth, Hirst and Plant, *J. Chem. Soc.*, 1214 (1935).



curacies discussed above; however, it seems safe to conclude that at most not more than 10% of non-reducing material of this type could be present, and probably none. The other possibility which must be considered is the presence of ring dextrins of the Schardinger type which have neither reducing nor non-reducing ends and would hence not be detected by the tetramethyl glucose analysis. However, this possibility is completely eliminated by the agreement of the rotations with the calculated values. The Schardinger dextrins have rotations about 20–25° lower than that calculated for an open chain of equal molecular weight, and their presence would have to be offset by low-molecular weight dextrins to bring up the reducing power; the net effect would be a marked lowering of the rotation of the fraction. Thus the suggestion that these materials consist of glucose, maltose and non-reducing dextrins is completely out of the picture, and the possibility of the presence of even a small amount of non-reducing material seems dubious.

The reaction of phenylhydrazine with cellulose dextrins has been investigated by Staud and Gray[17] and by Bergmann and Machemer,[15] who concluded that nitrogen analysis of the reaction product is more trustworthy than copper numbers as a basis for estimating molecular size. The application of the method to the starch dextrins was investigated in the hope of using it as another criterion of chain length, and also in the hope that the derivatives might possess some advantages for fractionation in that any non-reducing material would not react. The results indicated that the reaction is quantitative but that the derivatives are unstable. These conclusions were substantiated by the preparation of the derivatives of glucose and maltose.

Both hydrazide and hydrazone forms of the phenylhydrazine derivatives of glucose have been reported.[18] The glucose derivative obtained here was apparently a mixture of the two forms but predominantly the hydrazide. Obviously rather complicated equilibria are involved, but the following simplified mechanism would seem to explain the phenomena so far observed.

The equilibrium between the phenylhydrazide form (I) and the phenylhydrazone form (II) has been recognized.[18] The fact that a large amount of the phenylhydrazine is immediately released from the maltose derivative upon solution in water, followed by a slow hydrolysis of the remainder, would seem to indicate that the hydrolysis of the phenylhydrazide is quite rapid whereas the shift between the two tautomeric forms is slow. The hydrolysis is evidently inhibited by acid, as is shown by the fact that derivatives prepared using 50% acetic acid for purification were found to contain about the same per cent. nitrogen as the derivatives prepared by the modified method.

Figure 4 shows reasonable agreement of the per cent. nitrogen with the calculated values up to a molecular weight of about 1000, corresponding to about six glucose units. From this point on the per cent. nitrogen is much too high, indicating adsorption of phenylhydrazine by the carbohydrate. There is considerable evidence that the chains in starch and its derivatives are coiled in the form of a helix with a periodicity of about six

(17) Staud and Gray, *Ind. Eng. Chem., Anal. Ed.*, **1**, 80 (1929).

(18) For a review of these compounds see Tollens and Elsner, "Kurzes Handbuch der Kohlenhydrate," Fourth Ed., J. A. Barth, Leipzig, 1935, pp. 231–232.

glucose units.[19,20] If the adsorption of phenylhydrazine is due to entrapment in the center of these helices, it might be expected that no noticeable amount of adsorption[21] would occur until the average chain length of the dextrin was approximately six to eight glucose units, as is observed. It is conceivable that by varying the solvents used and by controlling the conditions of precipitation it might be possible to eliminate adsorption and to obtain the derivatives in the more stable hydrazone form.

Summary

1. A method for isolating the dextrins from corn sirup in large quantities is given.

2. Repeated alcohol fractionation gives fractions ranging in mean molecular weight from less than two glucose units to 26 units, and the higher fractions are concluded to be relatively free of maltose and glucose.

3. Oxidation of the dextrins to the potassium salts of the dextrinic acids can be carried out in good yields and the potassium content of the products checks with the reducing value of the original dextrin, indicating the iodine reaction to be quantitative and a true measure of molecular size.

4. The specific rotations of the dextrins agree with the values calculated from iodine molecular weights, lending further evidence to the reliability of iodine values.

5. The Freudenberg and Boppel method of methylation is found to be very satisfactory for both the dextrins and dextrinic acids.

6. Tetramethyl glucose assay of the methylated dextrins indicates the chains to be essentially unbranched, and non-reducing fractions to be absent. The former conclusion is substantiated by the almost complete absence of dimethyl glucose, the latter, by the agreement of the rotations with the calculated values.

7. The reaction of phenylhydrazine with the smaller starch dextrins is found to be quantitative, but the derivatives are unstable and postulated to be largely of the phenylhydrazide type.

8. The dextrin fractions averaging greater than about six glucose units in length show a strong tendency to adsorb phenylhydrazine and a possible explanation is given.

(19) Hanes, *New Phytologist*, **36**, 101, 189 (1937).

(20) Bear, THIS JOURNAL, **64**, 1388 (1942).

(21) The word is here used with the realization that neither the term adsorption nor absorption as used in the usual sense fits this phenomenon.

AMES, IOWA RECEIVED MAY 8, 1942

[CONTRIBUTION FROM THE BIOCHEMICAL LABORATORY, STATE UNIVERSITY OF IOWA]

Antioxidants and Autoxidation of Fats. XIV. The Isolation of New Antioxidants from Vegetable Fats[1]

BY CALVIN GOLUMBIC

The tocopherols have been found in a wide variety of vegetable fats but are generally absent from animal fats.[2,3] The observations of Olcott and Emerson[4] and later those of Golumbic[5] have shown that they and related compounds function as fat antioxidants and they are responsible in part for the greater stability of vegetable fats toward oxidative deterioration.

When added to animal fats that are exposed to air or oxygen, tocopherols are rapidly oxidized during the period in which they exert their antioxygenic action and their complete disappearance practically coincides with the end of the induction period of the fat.[6] At this point there is a readily detectable increase in the rate of oxygen uptake and of peroxide formation[6] (Table I). In an autoxidizing hydrogenated vegetable fat, on the other hand, the pronounced acceleration of peroxide formation does not occur until a considerable time after the total disppearance of tocopherol (Table I). This observation suggested the presence of hitherto unrecognized antioxidants which were less susceptible to oxidation than the tocopherols. They were obviously not phenolic in nature because the oxidized fat gave no test with the ferric chloride–dipyridyl reagent of Emmerie and Engel.[7]

In some respects, the oxidized vegetable fat behaved as though it contained quinoid substances.

(1) Presented before the Division of Food and Agricultural Chemistry, American Chemical Society meeting, Memphis, Tenn., 1942.

(2) Olcott and Mattill, THIS JOURNAL, **58**, 1627 (1936).

(3) Karrer and Keller, *Helv. Chim. Acta*, **21**, 1161 (1938).

(4) Olcott and Emerson, THIS JOURNAL, **59**, 1008 (1937).

(5) Golumbic, *ibid.*, **63**, 1142 (1941).

(6) Golumbic and Mattill, *ibid.*, **63**, 1279 (1941).

(7) Emmerie and Engel, *Rec. trav. chim.*, **57**, 1357 (1938).