other plant species. The cellulose is identical with the normal cotton cellulose.

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THE CHEMICAL COMPOSITION OF CORN (ZEA MAYS) SEEDLINGS. II. THE ISOLATION OF A DEXTRIN SIMILAR TO THE TRIHEXOSAN OBTAINED BY THE THERMAL DEPOLYMERIZATION OF POTATO STARCH

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Introduction

Dextrins are usually regarded as the intermediate products formed in the course of the hydrolysis of starch to maltose and glucose by diastatic enzymes.[2] Numerous dextrins formed in the course of the degradation of the starch molecule have been studied by various early workers;[3] recently more thoroughly by Samec[4] and Ling and Nanji.[5]

The occurrence of dextrins or dextrin-like substances in the growing plant has frequently been reported.[6] In no case, however, have the dextrins been isolated in a sufficiently pure state to permit definite characterization

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[1] This publication comprises part of a thesis submitted to the Graduate Faculty of the University of Wisconsin, in June, 1925, in partial fulfilment of the requirements for the degree of Doctor of Philosophy. The research was concluded during 1925–1927 while the author was a Fellow of the International Education Board posted at the University of Zürich, Switzerland, and the University of Graz, Austria. The author wishes to acknowledge his indebtedness to Prof. Paul Karrer, Director of the Institute of Chemistry, University of Zürich, for the privilege of extending the research while a student in his laboratory and for advice and help received. The author is likewise indebted to Professor Fritz Pregl, Director of the Medico-Chemical Institute of the University of Graz, under whose personal direction the micro-analytical analyses were conducted. To the Board of Directors of the International Education Board (New York City) the author extends his thanks for the Fellowship grant which enabled him to complete the work in the laboratories mentioned.

[2] (a) H. Pringsheim, "Die Polysaccharide," Springer, Berlin, **1923,** 2d ed., p. 155; (b) C. Brown, "Handbook of Sugar Analysis," John Wiley and Sons, Inc., New York, **1912,** pp. 685–690.

[3] Ref. 2 b, p. 685.

[4] Samec, *Kolloid-Chemie Beihefte,* **10,** 289 (1919).

[5] Ling and Nanji, *J. Chem. Soc.,* **123,** 2666 (1923); **127,** 629 (1925); **127,** 637 (1925).

[6] (a) F. Czapek, "Biochemie der Pflanzen," Fischer, Jena, **1922,** 3d ed., Vol. I, p. 441; (b) C. Wehmer, "Die Pflanzenstoffe," Fischer, Jena, **1911,** numerous citations.

and identification. The order of the molecular aggregation of the dextrins present in plant tissue may vary from $(C_6H_{10}O_5)_2$, the anhydride of maltose, to the high state of polymerization found in starch, $(C_6H_{10}O_5)_n$. It is also possible that many of the dextrins reported present in the growing plant belong to the cellulose dextrin series and represent intermediate compounds used in the formation of cell wall tissue.

In 1922 Pictet[7] reported the isolation of a trihexosan, $(C_6H_{10}O_5)_3$, obtained by the thermal depolymerization of potato starch in glycerin at the temperature of 200–210°. By heating potato starch in glycerin until the solution just failed to give a color reaction with iodine, and then quickly removing the solvent by vacuum distillation at the same temperature, a transparent, vitreous, light brown solid was obtained, soluble in water and precipitable with ethyl alcohol. Upon purification by reprecipitating with alcohol, the product was procured as a white, amorphous, slightly hygroscopic powder, molecular weight, 503; α_D^{20} + 162.2 in water. Sjöberg[8] reported the formation of a dextrin, by the action of amylose on amylopectin, identical with the trihexosan of Pictet. The same product was obtained by Pringsheim[9] by the action of glycerin on glycogen at the temperature of 200°.

Since 1922 it has been known[10] that the radicle and plumule of corn seedlings (Zea Mays) contain dextrins, varying in amounts from 1.25 to 3.00% of the total dry weight of the seedlings. The dextrin content was found to be highest in seedlings grown near the optimum temperature of growth, namely, at 24–28°. At these temperatures the quantity of dextrin present is usually double the value found in seedlings grown at 12–14°.

It was noted that the dextrins present in the seedlings grown at 24–28° could be roughly divided into two fractions. The fractions were both soluble in water and dilute alcohol (below 40%) but insoluble in alcohol of over 80% concentration. By adding sufficient alcohol to an aqueous solution of the dextrins to insure a concentration of 40%, one fraction was precipitated which gave a faint red color with iodine solution. By increasing the concentration of the alcohol to over 75%, a second fraction fell out, which in the crude state gave a faint yellow test with iodine. Enough of this second fraction was prepared to enable a study to be made thereupon. The dextrin fraction precipitated by 75% alcohol was found to be very similar to the trihexosan, $(C_6H_{10}O_5)_3$, obtained by Pictet. It comprises about 65% of the total dextrin content of the seedlings. The fraction precipitated by 40.0% alcohol is a complex mixture of higher dextrin aggregates, a report of which will be made later.

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[7] Pictet and Jahn, *Helv. Chim. Acta*, **5**, 640 (1922).

[8] Sjöberg, *Ber.*, **57**, 1251 (1924).

[9] Pringsheim, *ibid.*, **57**, 1581 (1924).

[10] Unpublished data of J. G. Dickson and K. P. Link.

Experimentation

Corn seedlings were grown at 24–28° by germinating them in soil, in the Wisconsin constant temperature soil tanks.[11] The seedlings, planted at a depth of 2.5 inches, were harvested when they reached the stage where the coleoptile ruptured the leaf sheath.[12] They were then removed from the soil, washed and separated from the remaining endosperm tissue. The method of killing plant tissue at 98°, followed by rapid drying at 65° in a current of air, previously reported by the writer, was used to preserve the tissue.[13] The dried tissue, containing approximately 6–8% of water was prepared for the chemical investigations in the identical manner used by the writer with other plant tissue.[14]

Extraction of Fat, Pigments, Waxy Constituents and Sugar.—Four kilos of the ground tissue was extracted in a large Soxhlet with ether for thirty-six hours and then with petroleum ether (b. p. 90–110°) for fifteen hours. After removing the fat solvents, the extraction was continued for twenty-four hours with 95.0% alcohol to remove the sugar present.

Extraction of Dextrins.—The fat, pigment and sugar-free residue was placed in a large bottle with 20 times its weight of 20.0% alcohol and extracted by shaking upon a machine for six days. The extraction was repeated for two days with a fresh portion of alcohol. No more dextrins could be removed with 20.0% alcohol after the second extraction. The large volume of 20.0% alcohol was then concentrated carefully in a vacuum of 15 mm. at 40° to one-seventh of the original volume. Alcohol (99.0%) was added until the concentration approximated 40.0%. The solution was then kept in an ice chamber at 2° for four days, during which time the separation of the dextrin fraction that gave a positive reaction with iodine was complete. After the precipitated dextrin was filtered, sufficient 99.0% alcohol was added to the filtrate to produce a concentration of 85.0%. On standing for five days at 2° the precipitation of the second dextrin was complete. The yield of the crude product was 72.0 g. This was purified by dissolving in water and reprecipitating with alcohol. After six reprecipitations the product was obtained as an amorphous snow-white powder, free from ash and nitrogen. The yield of the purified product was 63.0 grams.[15]

Properties and Analysis of the Dextrin.—The dextrin does not reduce Fehling's solution, gives no color with iodine and has a feeble insipid taste. It decomposes at 220–230° without melting. The specific rotation in water is $[\alpha]^{20}_{D}$, +163.6. The ultimate analysis showed C, 44.44; H, 6.19 (Pregl micro method); calcd. for $(C_6H_{10}O_5)_3$: C, 44.44; H, 6.21%. On hydrolysis in 2.5% sulfuric acid it was converted into glucose in 92.0% yields. The osazone prepared from the hydrolyzed solution showed m. p. 204–208°, the m. p. for glucosazone. The nono-acetyl derivative, $C_{36}H_{48}O_{24}$, prepared after the method of Pictet, showed m. p. 152–153°; $[\alpha]^{20}_{D}$, +125.9° in chloroform.

The dextrin (trihexosan) was converted into the monomethyl derivative, methoxyl content 17.40% (Zeisel–Pregl) in the manner employed by Kuhn and Zeise.[16] The

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[11] Jones, Johnson and Dickson, Wisconsin Agr. Expt. Sta., *Bull.*, **71** (1926).

[12] At this stage the seedlings are emerging from the soil. The photosynthesis of food materials has not begun. All the chemical constituents present in the seedlings are therefore derived from the embryo and endosperm reserves.

[13] Link, THIS JOURNAL, **47**, 470 (1925).

[14] Link, *ibid.*, **45**, 439 (1923).

[15] The product was dried first in 95.0%, then in 99.5%, alcohol. The alcohol was replaced by ether, which was in turn removed at 15 mm. pressure in a vacuum desiccator over calcium chloride.

[16] Kuhn and Zeise, *Ber.*, **59**, 2314 (1926).

monomethyl derivative gave no color with iodine and could not be saccharified with diastatic enzymes. Upon hydrolysis in 5% hydrochloric acid it gave 6-monomethylglucose, isolated as the osazone, m. p. 176–179°, identical with the figure given by Helferich and Becker.[17]

An attempt was made to determine the molecular weight of the unsubstituted product by the cryoscopic method, but consistent values could not be obtained. Using water as the solvent, the values found varied between 510 and 639. Pictet reported the molecular weight of 503 for the unsubstituted trihexosan and 871 for the mono-acetyl derivative. The molecular weight determinations of the mono-acetyl derivative varied between the values 820 to 960° with chloroform as the solvent and 790 to 980 with glacial acetic acid as the solvent.

Discussion

The properties and analyses of the dextrin isolated from the corn seedling indicate that it is quite comparable to the trihexosan, $(C_6H_{10}O_5)_3$, prepared by Pictet by the thermal depolymerization of potato starch in glycerin at 200–210°. It is prudent not to consider the dextrin from the corn seedling as strictly identical with the trihexosan of Pictet, since the molecular weight determinations are not in agreement with the figures reported by Pictet.[18]

The molecular weight determinations of polysaccharide compounds and their derivatives have long since been known to give contradictory results. Time and time again the chemist has been deceived by drawing rigid conclusions from molecular weight data obtained in the study of amorphous polysaccharide substances. The work of Karrer,[19] Bergmann,[20] Pringsheim,[21] Hess[22] and their co-workers has revealed the most unsuspected and unconquerable difficulties, due to the tendency of polysaccharide substances to form molecular aggregates through association. Molecular weight determinations of amorphous substances should be accepted with precaution, as Karrer has pointed out in his monograph.[23] Therein he states that he had failed to obtain the trihexosan of Pictet and the dihexosan of Pringsheim and Sjöberg in a true crystalline condition, as these workers had reported. Furthermore, Karrer questions the homogeneity of both the dihexosan and trihexosan. The argument is advanced that trihexosan can be hydrolyzed to maltose by enzymes, without the simultaneous formation of glucose, and that it can likewise be converted into acetobromo-

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[17] Helferich and Becker, *Ann.*, **440**, 1 (1924).

[18] It is not known whether the thermal depolymerization of corn starch yields a trihexosan identical with the trihexosan from potato starch.

[19] Karrer, *Ergebnisse der Physiol.*, **20**, 464 (1922).

[20] Bergmann, Knehe and Lippmann, *Ann.*, **458**, 93 (1927); **449**, 302 (1926); **452**, 141 (1927).

[21] Pringsheim, *Naturwissenschaften*, **12**, 360 (1924).

[22] Hess and Schultze, *Ann.*, **448**, 114 (1926); **455**, 84 (1927); **455**, 198 (1927).

[23] Karrer, "Die Polymere Kohlenhydrate," Akademisches Verlagsgesellschaft, Leipzig, **1925**, pp. 86–88.

maltose by the action of acetyl bromide, without the formation of acetobromoglucose. From these experiments Karrer concludes that the trihexosan, $(C_6H_{10}O_5)_3$, cannot be a three-membered glucose structure (that is, one molecule of glucose with one molecule of maltose), but that it must be a higher glucose polymer, which would yield maltose quantitatively upon hydrolysis and acetobromomaltose by the action of acetyl bromide. Karrer rejects the validity of the molecular weight determinations of the trihexosan and regards any conclusion drawn therefrom as presumptuous.

Pictet adhered to the belief that the trihexosan, $(C_6H_{10}O_5)_3$, represents the basal structural unit of the potato starch molecule, since it could be obtained in yields of the order of 90.0%. Sjöberg obtained both a dihexosan and a trihexosan from starch by enzymatic hydrolysis and concluded that the Pringsheim theory of the basal di- and trihexosan structure of starch was correct. According to this theory, the dihexosan is regarded as derived from the amylose and the trihexosan from the amylopectin of the starch grain. A dextrin comparable to dihexosan could not be isolated from the corn seedling. The dextrins present, besides the one closely related to Pictet's trihexosan, are of a higher molecular order.

Since the corn seedlings had not begun the photosynthetic process at the stage at which they were harvested, the dextrins in the tissue may be regarded as derived from one or both of two sources. They might have been translocated from the endosperm through the scutellum into the radicle and plumule—or they might have been synthesized from the sucrose present. Maltose appears to be absent from the radicle and plumule of the corn seedling at the stage of the rupturing of the leaf sheath by the coleoptile.

The chemical properties of the dextrin isolated (tabulated in Tables I and II) indicate that it is logical to regard it as a member of the starch dextrin series rather than of the cellulose dextrin series. Micheel,[24] one of Hess' co-workers, has recently isolated an amorphous trihexosan by the acetolysis of cellulose with hydrobromic acid and acetyl bromide. The physical constants and properties of the trihexosan isolated by Micheel indicate that it is not identical or related to the trihexosan obtained from starch.

It appears, therefore, that the dextrin isolated from the corn seedling represents a glucose polymer of the starch dextrin series, rather than of the cellulose dextrin series, and that it is not a polysaccharide substance directly used in the synthesis of the structural elements of the cell wall. It is possible that the dextrin is used as a respiratory material in the metabolism of the seedling.

In the following table the properties and physical constants of the dextrin isolated from the corn seedling are listed for comparison with those of

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[24] Micheel, *Ann.*, **456**, 69–86 (1927).

Pictet's trihexosan prepared from potato starch by thermal depolymerization and the Micheel–Hess trihexosan obtained from cotton cellulose by the action of hydrobromic acid and acetyl bromide.

TABLE I

COMPARISON OF PROPERTIES

Properties and constants	Dextrin from corn seedlings (Link)	Trihexosan from potato starch (Pictet)	Trihexosan from cellulose (Micheel-Hess)
Physical state	Amorph. white powder	Amorph. white powder[a]	Amorph. white powder
Soly. in water	Readily	Readily	Readily
M. p., °C.	Decomp. 220–230	Decomp. 230–232	Decomp. 184–189
Sp. rot. (water as solv.)	+163.6°	+162.2°	+90.5°
Mol. wt. (cryosc. meth., water as solv., calcd. for $(C_6H_{10}O_5)_3$, 486)	510–639	503.0	445[b]
Action on Fehling's soln.	None	None	None

[a] Reported as crystalline in the review by Pictet in Walton, "A Comprehensive Survey of Starch Chemistry," Chemical Catalog Co., New York, **1928.** In the original, *Helv. Chimica Acta,* **5,** 640 (1922), as amorphous.

[b] Micheel and Hess state that their trihexosan and its acetyl derivative do not exhibit association properties.

The properties and constants for the corresponding nona-acetyl derivative are listed in Table II.

TABLE II

COMPARISON OF PROPERTIES

Properties and constants	Nona-acetate, Link dextrin	Nona-acetate, Pictet trihexosan	Nona-acetate, Micheel-Hess trihexosan
Physical state	Amorphous	Amorphous	Cryst. add. compds. with solns.
M. p., °C.	152–153	153–154	123–126
Sp. rot.	+125.9° in $CHCl_3$	+125.8–126.1° in glac. AcOH	+86.6° in $CHCl_3$
Mol. wt. (cryosc. meth., calcd. for $C_{36}H_{48}O_{24}$, 864)	820–960 in $CHCl_3$, 790–980 in glac. AcOH	871 in $CHCl_3$	834[a] in glac. AcOH

[a] Micheel and Hess state that their trihexosan and its acetyl derivative do not exhibit association properties.

In concluding the author wishes to acknowledge his indebtedness to Dr. James G. Dickson, of the Department of Plant Pathology, for advice and criticism received throughout the course of this investigation.

Summary

A dextrin has been isolated from the radicle and plumule of corn seedlings, which appears to be similar to the trihexosan, $(C_6H_{10}O_5)_3$, obtained

by the thermal depolymerization of potato starch. The dextrin comprises approximately 65% of the total dextrin content of the corn seedlings. Since the dextrin belongs to the starch dextrin series, it appears probable that it is a polysaccharide substance used in the respiratory process of the seedlings.

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MONOARYLGUANIDINES. II. BENZOXAZOLEGUANIDINE[1]

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Introduction

Since *o*-aminophenol reacts with organic compounds containing the nitrile group to form benzoxazoles,[4] it was thought that this type reaction might be employed as an additional confirmation of the cyanoguanidine formula of dicyandiamide.[5] Dicyandiamide was first prepared by Beilstein and Geuther,[6] and the present name was given to the compound by Haag.[7] In addition to the Bamberger formula three other possible structural configurations have been suggested.[8] The Bamberger α-cyanoguanidine formula, originally suggested with very little substantial proof, is now generally accepted, and probably the most convincing arguments for the correctness of this formula have been advanced by Hale and Vibrans,[9] who reviewed the literature concerning dicyandiamide and studied the product of the reaction between acetylacetone and dicyandiamide. Blair and Braham[10] believe that this structural configuration explains the formation of biguanide and guanidine salts when dicyandiamide is fused

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[1] This paper was presented at the Columbus meeting of the American Chemical Society, April 30–May 4, 1929.

[2] This paper is constructed from part of the thesis submitted by Mr. Kane in partial fulfilment of the requirements for the degree of Bachelor of Science in Chemistry at the Polytechnic Institute of Brooklyn in June, 1928.

[3] Assistant Professor of Chemical Microscopy at Cornell University.

[4] (a) Skraup, *Ann.*, **419**, 75 (1919); (b) Wheeler, *Am. Chem. J.*, **17**, 400 (1895).

[5] Suggested by Bamberger, *Ber.*, **16**, 1074 (1883).

[6] Beilstein and Geuther, *Ann.*, **108**, 99 (1858); **123**, 241 (1862).

[7] Haag, *ibid.*, **122**, 22 (1862).

[8] (a) Strecker, "Lehrbuch der Organischen Chemie," 5th ed., p. 637, through Hallwachs, *Ann.*, **153**, 293 (1870); (b) Klason, *J. prakt. Chem.*, [2] **33**, 126 (1886); (c) Pohl, *ibid.*, [2] **77**, 533 (1908).

[9] Hale and Vibrans, This Journal, **40**, 1046 (1918).

[10] Blair and Braham, *ibid.*, **44**, 2342 (1922).