The phenylurethan, crystallized from petroleum ether, melted at 54°. This is a new compound.

*Anal.* Subs., 0.0608: AgCl, 0.0381. Calcd. for $C_{11}H_{14}O_2NCl$: Cl, 15.58. Found: Cl, 15.50.

The α-naphthylurethan prepared similarly and crystallized from petroleum ether melted at 69–70°. Bennett and Heathcoat give the melting point as 66°.

*Anal.* Subs., 0.3401: AgCl, 0.1768. Calcd. for $C_{15}H_{16}NO_2Cl$: Cl, 12.78. Found: Cl, 12.85.

## Summary

1. A new method of synthesis of tetramethylene glycol has been devised. Müller's method (published after this work had been completed), involving the reduction of diethyl succinate, appears to be cheaper, simpler and more rapid.

2. Tetramethylene chlorohydrin was isolated in a pure state for the first time; its physical properties were determined and its phenylurethan and α-naphthylurethan were made, the former being a new derivative.

HOUSTON, TEXAS

---

[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL CHEMISTRY, UNIVERSITY OF WISCONSIN, AND THE OFFICE OF CEREAL CROPS AND DISEASES, BUREAU OF PLANT INDUSTRY, UNITED STATES DEPARTMENT OF AGRICULTURE]

# THE CHEMICAL COMPOSITION OF CORN (ZEA MAYS) SEEDLINGS. I. THE ISOLATION OF XYLAN AND CELLULOSE FROM THE CELL WALLS[1,2]

BY KARL PAUL LINK

RECEIVED MARCH 23, 1929 PUBLISHED AUGUST 7, 1929

## Introduction

In the course of a general investigation on the nature of corn (Zea Mays) seedling blight, it was deemed expedient to investigate the chemical com-

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

[2] 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 St. Andrews, Scotland, and the University of Graz, Austria. The author wishes to acknowledge his indebtedness to Sir James Irvine, D.Sc., F.R.S., Principal and Vice Chancellor of the University of St. Andrews, 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 wishes to extend his thanks for the Fellowship grant received which enabled him to complete the work in the laboratories mentioned.

position of the seedlings with the purpose of correlating chemical composition with disease resistance. A preliminary report was made in 1923,[3] wherein the general purposes of the investigation were presented. This paper deals primarily with the studies made to determine the nature of the chemical constituents that compose the cell walls of the corn seedling.

It is of importance, and also of general interest to plant biochemistry, to know whether the environmental factor, temperature, can modify or alter the nature of the chemical components laid down in the cell wall. Of equal significance is the question, "Are the cell wall substances formed in the early stages of plant growth the same as those in the mature plant?" In cellulose chemistry the question frequently arises,[4] "Is there one true cellulose, $(C_6H_{10}O_5)_n$, universally distributed throughout nature, and made up of glucose units linked in an identical manner, or are there many types and kinds of cellulose?" The criterion commonly used to designate a true cellulose is the cotton fiber. Cotton is regarded as the standard example of a normal cellulose.[5]

The corn seedling lends itself very readily to a study of the general considerations mentioned. Very sensitive responses to the environmental factor of temperature are exhibited by the corn plant. At the optimum growing temperature, 24°, the rate of development of the seedlings is approximately four to five times that attained at the temperature of 12°. The cell walls of the corn seedling are relatively free from lignin. In the epidermal and endospermal layers there are some suberized and cutinized cells, but the incrustating substances are of such a nature that they can be readily removed. The necessity of applying strong oxidizing reagents to approach the study of the polysaccharide constituents is thereby eliminated. Nitrogenous substances of a very stable and resistant nature are likewise not found in the corn seedling. The nitrogenous compounds present can be readily removed without attacking the basic cell wall structure.

## Experimentation

The studies here recorded were conducted on two lots of corn seedlings produced at 12 and 24°.[6] The separate batches of seedlings were grown in soil in the Wisconsin

[3] Dickson, Eckerson and Link, *Proc. Nat. Acad. Sci.*, 9, 343 (1923).

[4] Irvine and Hirst, *J. Chem. Soc.*, **125**, 15 (1924); Heuser, "Lehrbuch der Zellulosechemie," Gebrüder Borntraeger, Berlin, **1927**, 3d ed.

[5] Hess, "Die Chemie der Zellulose und ihrer Begleiter," Akademische Verlagsgesellschaft, Leipzig, **1928**. This work also contains a very complete treatise on the chemical nature of the substances that accompany cellulose in nature.

[6] The results presented throughout the experimental part of this paper are those obtained by the study of seedling tissue grown at 24°. Tissue grown at 12° was studied in the same manner. The general analytical results obtained from comparable preparations of either 12 or 24° tissue were the same. The quantitative differences found are referred to in the discussion and will be presented in detail in a forthcoming publication.

constant temperature chambers[7] to the same stage of development. The seedlings planted at a depth of two and one-half inches were harvested when they reached the stage where the coleoptile ruptured the leaf sheath. At 24° five days are required, while at 12° the seedlings reach this stage in twenty to twenty-two days. After removing the soil by washing with water, the sprouts (radicle and plumule) were separated from the endosperm and immediately steeped in 95% alcohol.[8] A sufficient quantity of alcohol was used to keep the final concentration above 70% after dilution by the water from the tissue. The alcohol was subsequently boiled under a reflux condenser for five hours to kill the tissue.

**Preliminary Extraction to Remove Non-polysaccharide Substances.**—The alcohol in which the seedlings were preserved was filtered off at the pump. The tissue was then ground in a Nixtamal mill using alcohol as the liquid medium. It was subsequently transferred to a large Soxhlet, after Sando,[9] and extracted, first with 90% alcohol for twenty-four hours and finally with 99% alcohol for ten hours. After the alcohol imbibed by the tissue had been removed in a current of air at 25°, the tissue was ground in a drug mill and finally in a ball mill until all particles could pass through a 120-mesh sieve. It was then extracted for twenty-four hours with ether in a Soxhlet and finally with petroleum ether (b. p. 90–110°). The use of the fat solvents was found to be very advantageous since they removed wax and gum-like substances that had remained intact during the alcohol extraction.

**Extraction of Protein, Pectin and Incrustating Substances.**—After the removal of the fats, pigments and sugars, the tissue was digested for thirty-six hours in 1% ammonium hydroxide solution at 25°, and pressed free from the extraction liquors in a hand press. The excess ammonia was removed by washing with warm water (65°).

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

[8] In all of the investigations conducted on the nature of the cell wall constituents of the corn seedling, it was found advantageous to refrain from dehydrating the green seedling tissue by the direct application of heat. The seedlings were therefore killed by steeping them in alcohol. They were also preserved in this medium. The various fractions extracted from the cell walls were likewise never dehydrated above the temperature of 25° until most of the moisture (96–98%) had been removed through the use of organic solvents. Alcohol of successively increasing concentrations and then dry ether were used to dehydrate the preparations. By the application of these solvents a flaky and friable physical consistency was always secured in all of the products obtained. The products were also always remarkably free from discoloration.

If a freshly precipitated xylan preparation is dehydrated directly, either by the application of a current of air or in a vacuum desiccator over calcium chloride, a hard, cohesive, leathery, discolored mass is obtained, from which the last traces of water cannot be removed except by heating in a vacuum at 95° for an extended period. A xylan product prepared by direct dehydration has quite different solubility properties than one first dehydrated by the use of organic solvents. Thus a xylan preparation dehydrated directly over calcium chloride was found to be only partially soluble in hot water and required fourteen hours for complete solution in 1% sodium hydroxide, whereas a similar preparation first dehydrated in alcohol and ether and then over calcium chloride, was readily soluble in boiling water and went into solution in 1% alkali in one hour at 22°. A cellulose preparation dehydrated at 30° in a current of air could not be converted into the triacetate by the method used in this investigation. It was likewise only slowly soluble in Schweitzer's reagent. On the other hand, the cellulose preparations that had first been dehydrated by the use of organic solvents could be readily acetylated and dissolved rapidly in Schweitzer's reagent.

[9] Sando, *Ind. Eng. Chem.*, **16**, 1125 (1924).

This extraction was then followed by the chlorine dioxide–sodium sulfite treatment of Schmidt and Graumann,[10] to remove coloring matter and residual lignin- and cutin-like substances. After six treatments with an aqueous chlorine dioxide solution containing approximately 0.25% of the gas, followed successively by six treatments with 2.0% sodium sulfite, these reagents had no further effect. By washing copiously with water at 65° the free chlorine and sodium sulfite were removed from the tissue. The water imbibed by the remaining polysaccharide substances was removed by suspending the residue for twelve hours, first in 95%, then in 98%, and finally in 99.5% alcohol, after which the alcohol was removed by shaking in a large quantity of absolute ether for fifteen hours. By drying in a vacuum desiccator (15-mm. pressure at 25°) over calcium chloride, the ether and any traces of alcohol still remaining were removed. From the aforementioned extractions the final product obtained was a snow-white amorphous powder.

**Extraction of Xylans.**[11]—One hundred grams of the dry product prepared as above, containing 0.33% of ash and 0.67% of nitrogen, was successively extracted five times for twenty-four hours with one liter of 5.0% sodium hydroxide (carbonate free) by shaking on a machine in a glass-stoppered bottle containing small glass rods. A sixth extraction did not remove more material. To the combined alkaline extracts 95% alcohol was added until complete precipitation was produced. The xylan precipitate was allowed to settle, whereupon the major portion of the liquid could be siphoned off. It was found that the precipitated xylan was most efficiently washed free from alkali. by centrifuging, using 50% alcohol containing 10% acetic acid as the washing medium. It was then suspended in 95% alcohol for twelve hours and finally in 99.5% alcohol for twenty-four hours. The alcohol in turn was replaced by ether. Approximately 23.0 g. of xylan was obtained. This is equivalent to 8.5–9.0% of Xylan A, based on the original dry weight of the seedlings. The analysis and properties of this preparation designated as Xylan A are given below.

**Extraction of Xylan B.**—The cell wall material, intact after the separation of Xylan A, had an ash content of 0.37% and contained 0.36% of nitrogen. The quantity of xylan determined by the 12% hydrochloric acid distillation after Kröber and Tollens[12] was 11.0% (based on the dry weight of the Xylan A free residue). The residue freed from Xylan A was therefore subjected to a 10% sodium hydroxide (carbonate free) extraction at 60° in the identical manner described above. After four extractions no more xylan was removed. The xylan was precipitated, washed and dried as already described. The yield of Xylan B was approximately 3.0–4.0%, based on the original dry weight of the seedling tissue.

**Purification of Xylan A.**—Xylan A was purified by reprecipitating it from an ammoniacal copper hydroxide solution after a method similar to that employed by Hess.[13] Ten grams was dissolved in 1 liter of 25.0% ammonium hydroxide containing 10 g. of copper hydroxide. By shaking the solution for one hour practically complete solution was obtained. A small amount of flocculent matter remained which was

[10] Schmidt and Graumann, *Ber.*, **54**, 1860 (1921).

[11] The term hemicellulose was employed by C. Schulze, *Z. physiol. Chem.*, **16**, 387 (1892), to denote a substance similar in character to cellulose, which is easily hydrolyzed by weak acids. The Xylans A and B, extractable with 5 and 10% alkali, are likewise quantitatively removed by a 2.50% sulfuric acid hydrolysis. They are therefore hemicelluloses according to the Schulze classification. The writer prefers the more exact terminology, Xylan A and B, to the term "Hemicellulose."

[12] Kröber and Tollens, *J. Landwirtsch.*, **48**, 357 (1900); *Z. angew. Chem.*, **15**, 477 (1902).

[13] Hess, Messmer and Ljubitsch, *Ann.*, **444**, 287 (1925).

removed by centrifuging. This material had a nitrogen content of 8.0%. It was therefore not further considered since it was undoubtedly of a proteinaceous nature. To the clear solution obtained after the removal of the afore-mentioned nitrogen-containing substance, glacial acetic acid was added until the solution was just faintly alkaline. The xylan was thereby precipitated, centrifuged and washed, first with 50% alcohol containing 10.0% of acetic acid to remove the copper, and finally with 95.0% ethyl alcohol to remove the acetic acid. The water was removed with alcohol and the alcohol with ether as stated above.

**Properties of Xylan A.**—The xylan product obtained consisted of a fine, snow-white amorphous powder, free from nitrogen[14] and contained only 0.22% ash. It is soluble in hot water and forms a thin jell on cooling which exhibits marked swelling properties when alcohol is added. In weak alkalies it is readily soluble at room temperature and can be quantitatively thrown out of solution by the addition either of acidified alcohol or glacial acetic acid. It does not reduce Fehling's solution and is levorotatory. The specific rotation (2-dcm. tube) in 1% sodium hydroxide lies between the values −80.00 and −83.86° (the specific rotation of the various xylans so far isolated varies between the values −70 to −85°).[15] The ultimate analysis showed C, 45.23; H, 6.09 (Pregl-micro method). Calcd. for xylan, $(C_5H_8O_4)_n$, 132.06: C, 45.44; H, 6.11. Distillation with 12% aqueous hydrochloric acid gave an amount of furfural estimated as the phloroglucide compound corresponding to 94.6% of xylan. In view of the later experiments reported, which indicate the absence of other pentoses, the figures quoted refer to pentosans in the form of xylan.

**Hydrolysis of Xylan A.**—Two grams of Xylan A corrected for ash and moisture were treated with 10 cc. of 72.0% sulfuric acid at 15° for two hours. Within this time solution was invariably complete. After adding sufficient water to produce a 2.0% acid solution, the solution was heated under a reflux condenser for two hours. In some of the preparations a very small amount of flocculent material resisted hydrolysis. After cooling, the solution was neutralized with the requisite quantity of barium carbonate and filtered at the pump. The barium precipitate was extracted twice with boiling water, the washings were combined with the original filtrate and concentrated to a sirup in a vacuum of 15 mm. at 45°. The sirup was taken up with water, filtered free from a small inorganic residue, clarified with activated blood charcoal, again filtered and finally made up to a volume of 100 cc. The reducing value of 10-cc. aliquots was determined by the Shaffer and Hartmann[16] method: found, 93.30%, calculated as xylose. Arabinose was absent from the hydrolyzed solution, since the di-phenylhydrazine test of Neuberg[17] was negative. The phenylosazone of xylose, m. p. 154–159 (from alcohol),

[14] The preparations are designated nitrogen-free on the basis of the macro-Kjeldahl method. Ridge, *Textile Institut*, **15**, 94 (1924); *C. A.*, **18**, 1752 (1924), has shown that when the nitrogen content of supposedly nitrogen-free cotton fibers (fibers subjected to the bleaching and weak alkali washing process) is determined by a method capable of detecting a nitrogen content of the low order of 0.001%, the fibers still contain approximately 0.035% of nitrogen. Nitrogen determinations made by the Pregl-micro Kjeldahl method on the various xylan and cellulose preparations reported in this paper never revealed a significant nitrogen content. The highest nitrogen value found was in a cellulose prepared from seedlings grown at 12° wherein the nitrogen content was 0.053% (Pregl-micro Kjeldahl method). It is therefore reasonable to regard the preparations as practically nitrogen free.

[15] B. Tollens, "Kurzes Lehrbuch der Kohlenhydrate," Barth, Leipzig, **1914**, 3rd ed., p. 475.

[16] Shaffer and Hartmann, *J. Biol. Chem.*, **45**, 365 (1921).

[17] Neuberg and Wohlgemuth, *Z. physiol. Chem.*, **35**, 40 (1902).

was obtained by treating a portion of the hydrolyzed solution with phenylhydrazine and sodium acetate. No mannose phenylhydrazone separated. The crude osazone precipitate obtained was completely soluble in dry acetone, which indicates that the osazone of glucose was not present. As a means of further identification, the Bertrand test for xylose was applied.[18] The characteristic boat-shaped needles of the double cadmium salt, $(C_5H_9O_6)Cd_2 + CdBr_2 + 2H_2O$, were obtained. The xylose was also procured in the free state by taking up the sirup from the hydrolysis of 5 g. of the xylan in enough absolute alcohol to insure complete solution. The alcohol was then concentrated until turbidity set in, whereupon glacial acetic acid was added. On seeding with an authentic specimen of *d*-xylose and standing for forty-eight hours in an ice chamber, the xylose crystallized out; yield, 3.96 g., 70.0% of the theoretical; m. p. 140–143°, $[\alpha]_D^{20}$ in water, +18.1°.

The above critical examination of the fraction extracted with 5% sodium hydroxide, designated as Xylan A, shows conclusively that it is an authentic xylan, uncontaminated with either arabinose, mannose or glucose.

**Purification, Properties and Analysis of Xylan B.**—Xylan B was purified and analyzed in the identical manner prescribed for Xylan A. The general properties were much the same. Xylan B was found to be free from arabinose, mannose and glucose. The ultimate analysis was in agreement with the theoretical value for $(C_5H_8O_4)_n$, the furfural yield was 93.5% and the percentage of xylose determined by reduction after the acid hydrolysis was 91.2%. The furfural and reducing value are slightly lower than those reported for Xylan A. The specific rotation in 1% sodium hydroxide was —79.20°. The amount of flocculent material that resisted the sulfuric acid hydrolysis was appreciably more than that of Xylan A. This undoubtedly accounts for the lower furfural and reducing values as well as the lower specific rotation.

**Isolation of Pure Cellulose, $(C_6H_{10}O_5)_n$.**—The cellular residue still intact after the 10% sodium hydroxide extraction was washed free from alkali, first with 50% alcohol containing 10% acetic acid and subsequently with 65% alcohol. It was then dried in alcohol and ether. The product was free from nitrogen and contained 0.69% of ash, mostly silicon dioxide. Although no more pentose-yielding extracts could be obtained with alkali, the product still gave considerable furfural when subjected to the 12% hydrochloric acid Kröber-Tollens distillation. The yield of furfural varied between 2.3 and 4.6%. The residue also liberated carbon dioxide when heated with 12% hydrochloric acid after the method of Nanji, Paton and Ling.[19] The furfural-yielding substances present, *which are not true pentosans*, belong either to the class of cellulosic compounds that Hägglund[20] terms furfuroids or are similar to oxidized celluloses. Heuser and Stöckigt[21] reported that the so-called oxidized cellulose liberates carbon dioxide when heated with dilute hydrochloric acid. The possibility that the carbon dioxide and furfural might come from glucuronic acid residues as Schmidt and coworkers[22] have found must also be considered.

To remove these ill-defined and poorly characterized substances it was necessary to subject the residue remaining after the Xylan B extraction to two two-hour treatments at 30° with 15% sodium hydroxide. The characterization of the substances extracted with the 15% alkali is now in progress. Due to the present lack of concise information on this class of substances, a detailed discussion will be deferred. The residue remaining after the 15% sodium hydroxide extraction was washed free

[18] Bertrand, *Bull. soc. chim.*, [3] **5**, 556 (1891).

[19] Nanji, Paton and Ling, *J. Soc. Chem. Ind.*, **44**, 253T (1925).

[20] Hägglund, "Holzchemie," Akademische Verlagsgesellschaft, Leipzig, **1928**, p. 73.

[21] Heuser and Stöckigt, *Celluloschemie*, **3**, 61 (1922).

[22] E. Schmidt, K. Meinel and E. Zintl, *Ber.*, **60**, 503 (1927).

from alkali and dried in the manner previously described. Fifty grams of a xylan-free residue yielded approximately 40.0 g. of a white, amorphous product. The cellulose prepared from corn seedlings, which had first been extracted with alkali until all xylan, furfuroid and oxidized cellulosic material had been removed, displays the important chemical properties of cotton cellulose.

**Analysis of the Cellulose.**—The ash content was 0.86% (silicon dioxide). It was free from nitrogen. The ultimate analysis showed C, 44.40; H, 6.15. Calcd. for $(C_6H_{10}O_5)_n$: C, 44.44; H, 6.21. With the zinc chloriodide reagent the cellulose preparation gives the characteristic violet color. It is completely soluble in ammoniacal copper solutions (Schweitzer's reagent) and can be quantitatively reprecipitated by the addition of acetic acid.[23] When subjected to the 12% aqueous hydrochloric acid distillation of Kröber and Tollens the cellulose yielded only traces of furfural. Only minute quantities of carbon dioxide are formed in the same distillation determined by the method of Nanji, Paton and Ling.

The triacetate, $(C_6H_7O_5(CH_3CO)_3)_n$, was prepared after the excellent method of Barnett, as modified by Irvine and Hirst[24] for the preparation of esparto cellulose triacetate. It was snow-white in color and differed from cotton cellulose acetate prepared in the same manner by being more flaky and softer in texture. The specific rotation of a chloroform solution ($c = 1.3$) was $-22.1°$; moisture content, 1.08%; ash, 0.28%; C, 49.85; H, 5.35 (Pregl-micro method). Calcd. for C, 50.00; H, 5.55. The acetyl value calculated as acetic acid was 62.2%. The theoretical value for cellulose triacetate is 62.51%.

The yield of $\alpha$-cellobiose-octa-acetate, $C_{12}H_{14}(COCH_3)_8O_{11}$, obtained by subjecting the cellulose to a simultaneous acetylation and hydrolysis varies between the values of 46 and 48% after the method of Hess[25] and 38 to 40% after the method of Freudenberg.[26] After purification according to the method used by Hudson,[27] the octa-acetate showed m. p. 223° and specific rotation in chloroform +41.6°. The accepted values are m. p. 221.5–222°, $[\alpha]_D^{20}$ in chloroform +41.3°. The yield of glucose, obtained as the methylglucoside, $C_6H_{14}O_6$, using the method of Irvine and Hirst, was 90.0% of the theoretical. The methylglucoside showed a permanent specific rotation of +157.2° in water and the m. p. 165–166°. Its methoxyl content was 7.68% (Pregl-micro Zeisel method); theoretical: $OCH_3$, 7.77. The only osazone detectable after hydrolysis with sulfuric acid, as described under Xylan A, was the osazone of *d*-glucose m. p. 204–208°. Xylose, mannose and galactose (by mucic acid method) were absent.

## Discussion

The cell walls of the corn seedling contain xylan, $(C_5H_8O_4)_n$. The total xylan content as determined by the furfural distillation and estimated as the phloroglucide compound varies between 10–12% of the original dry weight of the tissue. The xylan can be fractionated. Approximately 85–90% of the xylan is extractable with 5% sodium hydroxide (Xylan A), the remainder can be extracted with 10% sodium hydroxide (Xylan B).

In their purified state the two xylans, A and B, are snow-white amor-

[23] The specific rotation values of the corn seedling cellulose in alkaline copper solutions determined after the method of Hess, *Ann.*, **466**, 1 (1928), will appear in a subsequent publication.

[24] Irvine and Hirst, *J. Chem. Soc.*, **121**, 1585 (1922); **125**, 15 (1924).

[25] Hess, *Ann.*, **296**, 309 (1925).

[26] Freudenberg, *Ber.*, **54**, 767 (1921).

[27] Hudson and Johnson, THIS JOURNAL, **37**, 1276 (1915).

phous powders, soluble in boiling water and readily soluble in 1% sodium hydroxide. The chemical analysis and a study of their properties showed them to be essentially identical. They differ from each other in their initial solubility and in their behavior toward the zinc chloriodide reagent. This color test was until recently regarded to be specific for true cellulose.[28] One of Hess' co-workers[29] was the first to show that it was not specific for cellulose. Lüdtke found that a mannan isolated from the ivory nut gave a positive test with the reagent. Hess and Lüdtke[30] have also reported that a xylan from bamboo stalks gives a positive test, whereas a xylan isolated from the sulfite liquors of pine wood did not give the test. The Xylan A isolated from the corn seedling gave no color with the reagent, whereas Xylan B gave the typical violet color, formerly prescribed as specific for cellulose. A satisfactory explanation for this difference in apparently similar xylans is wanting. The ultimate composition agreed with the calculated value for $(C_5H_8O_4)_n$. The specific rotation determined in 1% sodium hydroxide varied between the values $-79.20$ to $-83.00°$. In each case the osazones obtained after hydrolysis with acid were identical under the microscope and melted at 154–160° without decomposition. The recrystallized xylose showed the correct initial and permanent specific rotation of $[\alpha]_D^{20} + 18.0$ and melted at 140–143°.

Although the xylans prepared from corn seedlings grown at 12 and 24°, respectively, are identical chemically, there is a quantitative difference in the distribution of the xylan fractions. The seedlings grown at 12° contain 10–15% more Xylan A than the 24° seedlings. Xylan B varies proportionally, being lower in the 12° seedlings than in the 24° seedlings. The total xylan content of the seedlings is approximately the same at either temperature. Chemically the xylan of the corn seedling is identical with the xylan of the mature corn plant (forthcoming paper). Its properties are similar to those assigned by other workers to xylans from various sources.[31]

About 7% of the furfural-yielding substances in the corn seedling are not true pentosans. These substances appear to be similar to the poorly characterized oxidized celluloses and furfuroids (see Hägglund, "Holzchemie" and Hess, "Chemie der Zellulose." They can be brought into solution by the use of 15% sodium hydroxide. In a forthcoming paper these substances will be discussed.

It has also been shown that the cell wall of the corn seedling contains pure

[28] C. van Wisselingh, "Die Zellmembran," Gebrüder Borntraeger, Berlin, **1926**, p. 45.

[29] Lüdtke, *Ann.*, **456**, 201 (1927).

[30] Hess and Lüdtke, *ibid.*, **466**, 23 (1928).

[31] Wheeler and Tollens, *ibid.*, **254**, 304 (1889); Tollens, *Ber.*, **23**, 2990 (1890); Thomsen, *J. prakt. Chem.*, **19**, 146 (1879); *Ber.*, **13**, 2168 (1880); **40**, 136 (1881); Hess and Lüdtke, *Ann.*, **466**, 18 (1928); **466**, 27 (1928); O'Dwyer, *Biochem. J.*, **17**, 501 (1923).

cellulose. Approximately 14–18% of the dry weight of corn seedlings is composed of authentic cellulose possessing the formula $(C_6H_{10}O_5)_n$ and convertible quantitatively into glucose on hydrolysis. The cellulose in corn seedlings, when freed from xylan and furfuroids by alkali, displays the essential chemical properties of cotton cellulose. The ultimate analysis agreed with the theoretical value of $(C_6H_{10}O_5)_n$; it is soluble in Schweitzers reagent and can be quantitatively reprecipitated. The triacetate was identical with cotton cellulose triacetate prepared in the same manner (see Table I). Hess ("Chemie der Zellulose," p. 391) has shown that cellulose acetate preparations obtained by various methods can be regarded as chemically identical even though they show some variance with respect to the physical properties like solubility, swelling properties, viscosity in solution, and film-building capacity. The variations are presumably due to the phenomenon of molecular association so commonly exhibited by solutions of polysaccharide substances. The corn seedling cellulose can also be converted into cellobiose-octa-acetate in yields comparable to those obtained with cotton cellulose. Corn seedlings produced at 24° contain approximately 4% more true cellulose than the 12° seedlings, but the cellulose in both cases is identical.

Physiologically it is significant that cell walls of four-day-old corn seedlings grown at 24° and twenty-day old seedlings grown at 12° (same stage of anatomical development) contain the same xylan, $(C_5H_8O_4)_n$, and the same cellulose, $(C_6H_{10}O_5)_n$, found in mature corn plants and in mature plants of other species. In the cell walls of the corn seedlings the basic structural cellulose has intimately associated with it the pentosan xylan. Xylose usually accompanies glucose in nature since these two sugars are structurally related. How the xylan is actually associated with the cellulose is unknown.

The researches of Irvine and Hirst[4] have illustrated the intimate association of xylan and cellulose in esparto grass. Hess and Lüdtke[30] have reported the simultaneous occurrence of xylan and cellulose in pine wood. Lüdtke[32] has found the same to be true in bamboo stalks. Hägglund and co-workers[33] have likewise recorded the accompaniment of xylan with cellulose in pine wood.

Through the brilliant contributions of Haworth,[34] Hirst,[35] Levene,[36] and others we now know that in their stable forms *d*-glucose and *d*-xylose have the 1,5-oxide ring structure. From Haworth's work[37] it is easy to perceive

[32] Lüdtke, *Ann.*, **466**, 27 (1928).

[33] Hägglund, Klingstedt, Rosengrist and Urban, *Z. physiol. Chem.*, **177**, 248 (1928).

[34] Haworth, Charlton and Peat, *J. Chem. Soc.*, **127**, 85 (1926).

[35] Hirst, *ibid.*, **127**, 350 (1926).

[36] Levene and Meyer, *J. Biol. Chem.*, **60**, 167 (1924); Levene and Simms, *ibid.*, **68**, 737 (1926).

[37] Haworth, *J. Soc. Chem. Ind.*, **28**, 295T (1927); *Helv. Chim. Acta*, **11**, 534 (1928).

how *d*-glucose through oxidation and subsequent decarboxylation of the resulting uronic acid could give rise to *d*-xylose.

Lüdtke has demonstrated that the cellulose in the ivory nut[29] and in bamboo stalks[32] is a true cellulose. To this list can be appended the cellulose of the corn plant. It appears that there is one true cellulose, $(C_6H_{10}O_5)_n$, universally distributed in the plant world.

TABLE I

COMPARISON OF THE PROPERTIES OF CELLULOSE TRIACETATE PREPARED FROM CORN SEEDLINGS WITH COTTON CELLULOSE TRIACETATE PREPARED BY VARIOUS METOHDS

| Cellulose and method of preparation | % AcOH, calcd. 62.51% | Spec. rot. in $CHCl_3$ | M.w. in glac. AcOH | Visc. of 20% $CHCl_3$ soln. | Film properties |
|---|---|---|---|---|---|
| Cotton cell. acet. in benz.[a] | 62.51 | −22.06 | 280 | Viscous | High elast. |
| Same acet. in pres. of $ZnCl_2$[a] | 62.61 | −22.62 | 272 | Viscous | High elast. |
| Same acet. with $CH_3COBr$[a] | 62.22 | −19.50 | 280 | Sl. visc. | Low elast. |
| Same acet. by Barnett meth. | 62.60 | −21.50 | Not. det. | Viscous | High elast. |
| Corn cell. by Barnett meth. | 62.60 | −22.10 | Not. det. | Sl. visc. | Low elast. and brittle |

[a] Cited from Hess, "Die Chemie der Zellulose," p. 392.

The author wishes to acknowledge his indebtedness to Dr. James G. Dickson, Department of Plant Pathology, and Prof. W. E. Tottingham, Department of Agricultural Chemistry, for advice and criticism received throughout the course of the investigation.

## Summary

1. Corn seedlings grown at 12 and 24° to the stage where the coleoptile ruptures the leaf sheath contain 10–12% of xylan, $(C_5H_8O_4)_n$, and 16–18% of $(C_6H_{10}O_5)_n$ cellulose.

2. The xylan can be fractionated, 85–90% can be extracted with 5% sodium hydroxide (Xylan A) and the remainder with 10% sodium hydroxide (Xylan B).

3. In the pure state the Xylans A and B are soluble in hot water, are strongly levorotatory and can be quantitatively hydrolyzed into *d*-xylose.

4. The hexose cellulose is composed entirely of glucose residues, is convertible into cellulose triacetate, into cellobiose-octa-acetate and into the methyl glucoside. In these respects and also in its high molecular complexity the compound resembles the cellulose of cotton.

5. No sugars other than xylose and glucose have been detected in the hydrolysis products obtained from the cell wall constituents of the corn seedling.

6. Temperature does not alter the qualitative nature of the xylan and cellulosic constituents laid down in the cell walls, but alters slightly their quantitative distribution.

7. The xylan of the corn seedling is similar to the xylans obtained from

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

DEPARTMENT OF AGRICULTURAL CHEMISTRY
MADISON, WISCONSIN

---

[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL CHEMISTRY, UNIVERSITY OF WISCONSIN, AND THE OFFICE OF CEREAL CROPS AND DISEASES, BUREAU OF PLANT INDUSTRY, UNITED STATES DEPARTMENT OF AGRICULTURE]

# 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

BY KARL PAUL LINK[1]

RECEIVED MARCH 23, 1929 PUBLISHED AUGUST 7, 1929

## 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

[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.