The Structure of the Cells of Vegetable Fibers

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B. Chemical Constitution of Vegetable Fibers

1. Method for Separating the Carbohydrates in the Cell Wall

The cell wall is, as has been pointed out, not chemically uniform. Besides cellulose, it mainly contains carbohydrates like mannans and xylans (wood gums), substances of the primary layer and the inner membranes, of which only few had been defined exactly. Also, the place where they are deposited was mostly unknown. It was found only recently that a method most frequently used for spotting cellulose, namely the coloration with chlorzinc iodine, is not specific for cellulose and that, for instance, mannan B and xylan of bamboo and beech wood also give this reaction.

The method for isolating and identifying the hemi-celluloses, which was worked out on the cell wall substance of the seeds of the Brazil nut and the wood of bamboo, and which then was applied on a practical scale to sulphite pulp and to beech wood, consists in treating the gently but completely macerated pulp with cold caustic soda of different concentrations so that it becomes extracted in fractions each of which contains only a few components from which the dissolved substances are separated with acetic acid and methanol.

The extracting of the hemi-celluloses begins already when a concentration of caustic of 1 to 2% is applied but is as a rule not yet completed with caustic of 8% when the dissolving of the initial amounts of cellulose sets in[26]. (Shaking with caustic at room temperature during 6 to 12 hours under exclusion of the air.) The fact that a lye of a certain concentration even when applied repeatedly extracts only part of a carbohydrate may be traced on the one hand to the organized structure of the cell wall as a system of tiny compartments, and on the other hand to the particular arrangement of certain structural units known as micelles.

The fractions obtained are dissolved in ammonia in the presence of hydroxide of copper and the individual carbohydrates are separated from the solution by additions of reagents which precipitate selectively. For this purpose the copper alkali-compounds have proven most useful, as it developed that the various substances of the cell wall precipitate with different concentrations of alkali and can be isolated. For instance, mannan A and B of the seed of Brazil nuts and the mannan of the wood of conifers, identical with A, precipitate (under certain conditions) in an alkali concentration of the total solution of 0.2π,[27] cellulose at 0.4 to 0.5π, xylan of bamboo wood, of beech wood, and of spruce wood at 1π[28], while a newly discovered glucoside in beech wood does not yet precipitate at this concentration but can be separated only after an addition of methanol.

Usually caustic soda was not used alone as a precipitating agent but in combination with acetic acid, alcohol, soda ash, and other reagents. After decomposition of the copper compounds with acetic acid and rinsing there remain white powders which after re-precipitating and treating with alkali represent pure substances. The effect of the purification is checked polarimatrically either in alkaline solution or in ammoniacal copper solution according to Hess and Messmer[29]. It is continued until a constant rotation is obtained.

[26] M. Lüdtke, A. 456, 214 (1927); K. Hess, M. Lüdtke, H. Rein, A. 466, 58, 1928.

[27] M. Lüdtke, A. 456, 201, 1927; K. Hess and M. Lüdtke, A. 466, 18, 1928.

[28] M. Lüdtke, A. 466, 27, 1928; K. Hess, M. Lüdtke and H. Rein, A. 466, 58, 1928.

[29] A. 435, 7, 1923.

Then the substance is examined for uniformity by dissolving it in various solvents and precipitating it with different reagents. If the substance now maintains the same degree of rotation, it is considered as uniform, and the sugar in it is determined quantitatively. An examination of the properties of the sugar derived completes the operation.

Now, it was found that the carbohydrates of the cell walls are built up from one sugar only. Manno-celluloses, as they have been described in the seeds of Brazil nuts and in the cellulose of sulphite pulp, could not be detected. Furthermore, the chemical distribution of substances largely corresponds to the anatomical distribution of structural elements. To consider the cell membrane as a mixture of different substances is not a sound theory, it is organized also from a chemical point of view.

2. Determination of Cellulose

The methods for determining cellulose which have been in use so far are not free from uncertainties. The indirect methods, which are based on the determination of the glucose, are unreliable for the reason that other bodies yielding glucose[30] may also be present, and make the determination of glucose itself a difficult operation. In those cases where the content of non-cellulose substances is ascertained, the difference being accounted for as cellulose, no accurate result can be obtained because of the inexactness of the methods used for this purpose.[31] Further, there remains the possibility that still unknown substances in small quantities may be present which are not taken into consideration.

The direct methods, on the other hand, are lacking a procedure which permits to prove that the substance obtained actually represents cellulose and not raw fiber.

These circumstances prompted us to search for means which enable the investigator to precipitate the unchanged cellulose from a complete solution of the cell wall, and to determine it directly. As a solvent, ammoniacal copper oxide came into consideration. The method of procedure which was at first tried on two samples was developed in the following manner: In the case of the seeds of Brazil nuts where only mannans and cellulose are present, the former were separated from the copper solution with a certain amount of alkali and then the cellulose was precipitated with acetic acid. The content of cellulose was 5.5%. In the pulp from bamboo xylan is present besides cellulose. The incomplete precipitation of the xylan at the addition of acetic acid was utilized to effect the separation of the cellulose. It was necessary to re-precipitate four times from ammoniacal copper oxide before the specific rotation value for cellulose could be obtained. In this way the cellulose content of bamboo pulp was found to be 70%, the remainder consisting mostly of xylan.

These experiments have not yet been concluded but they show that the possibility exists to arrive at exact cellulose determinations on this basis.

3. Substance of the Primary Layer

When bamboo or other vegetable fibers which underwent no other treatment but digesting are immersed in alcoholic hydrochloric acid, which contains a little phloroglucin,[32] and allowed to stand for about two days in the dark, the fibers assume a reddish violet coloration. This reaction is not caused by any remaining lignin, for an aqueous solution of phloroglucin does not produce the coloration. Besides, the fibers were completely soluble in sulphuric acid 70%.

The coloration did not appear when cotton was used (the cuticle of which consists of a wax-like substance) or pure cellulose, xylan, and mannan. Pulp after furfurol destillation and after extraction with caustic lye of 17% or stronger also did not give the reaction.

It can thus be shown that the known substances, cellulose, xylan and mannan can not cause this reaction but that another yet unknown substance is the reason for it. It can further be shown that with the disappearance

[30] K. Hess, M. Lüdtke, H. Rein, A. 466, 58, 1928. A glucosan was newly discovered in digested beech sprouts. See also E. Haegglund, F. W. Klingstedt, T. Rosenquist, H. Urban, H. 177, 248, 1928, who found glucose in the wood of spruce and from it conclude the presence of glucosan.

[31] See also E. Haegglund and F. W. Klingstedt, *Zellulosechemie* 5, 57, 1924. About wood gum (xylan) and pentose determination see A. 466, 27, 1928, as well as p. 18, etc.

[32] Saturate alcohol 98% at 6° C. with gaseous hydrogen chloride, add an equal amount of alcohol, and add before use 2 to 3 grams phloroglucin per 100 cc.

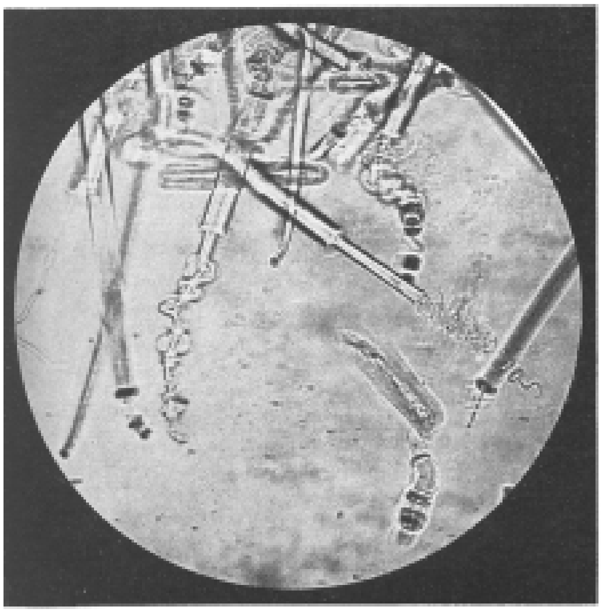


Figure 1
Bamboo Fiber X200 Magnification
Formation resembling string of beads takes place in every layer on account of tangential inner skins and transverse elements.

of the characteristic color reaction the typical swelling in ammoniacal copper oxide, described in the first part of this article, does not take place. The reaction, therefore, must be caused by the substance of the primary layer or cuticle.

In the following table the color reactions of some vegetable substances in alcoholic phloroglucin—hydrochloric acid and their swelling reaction in ammoniacal copper oxide are listed.

The table indicates how, with the disappearance of the primary layer by alkaline treatment or bleaching, reaction and swelling continuously become weaker.

When the pulp in moderate phloroglucin—hydrochlorin acid is heated, the primary layer turns red like a pentosan, at prolonged boiling it turns greenish brown. It appears, therefore, justified to ascribe to it groups which yield furfurol. Schwalbe and Feldtmann[34] have shown in a notable paper that glucuronic acid is present in pulp to the extent of a few per cent. But the attempt to separate the substance from bamboo pulp according to the directions of the author could, due to the lack of material, only qualitatively increase the probability that uronic acids or their anhydrides are present on the strength of the naphto-resorcin reaction which was obtained.

Since the substance disappears by treatments with mercerizing liquors and bleaching agents, it must be found in such liquors besides the hemi-celluloses. Many difficulties experienced in technical processes, especially dyeing, and in the manufacture of rayon can be traced to its presence.

4. The Structure

After what has been said in the preceding paragraphs, the structure of the vegetable fiber may be visualized in the following terms: A substance of furid character (in the case of vegetable hairs a wax-like matter) envelopes the entire fiber as the thin primary layer. Underneath it is the secondary layer which is often subdivided. The division is caused by delicate membranes imbedded tangentially in the secondary layer. The individual segments can be split into spiral layers and these into

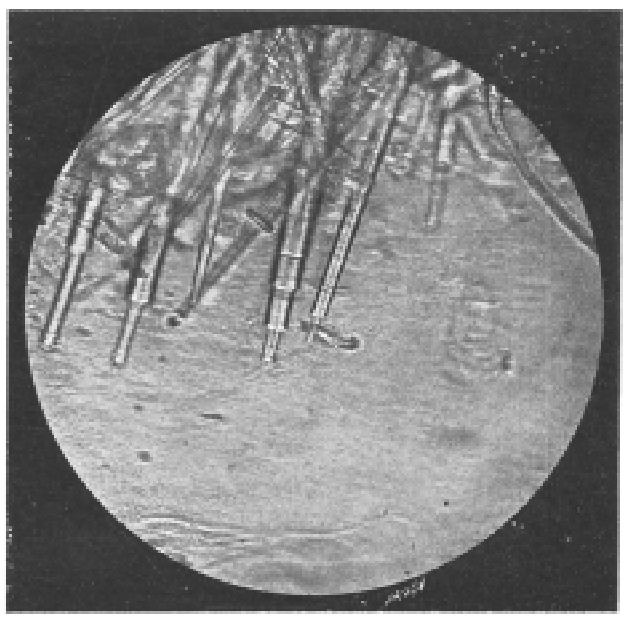


Figure 2
Bamboo Fiber X200 Magnification
Setback formation caused by presence of transverse elements.

fibrils. The foreign matter could so far not be detected between spiral layers and fibrils. But its presence may be assumed for reasons

[34] B. 58, 1534, 1925.

Substance and Its Previous Treatment	Hydrochloric Acid		Swelling in Ammoniacal Copper Oxide
	Alcoholic	Aqueous	
Bamboo Pulp: ClO_2, Na_2SO_3	33) + +	....	Good
2 times extracted with 8% NaOH	+	....	Occasional
22 days in 18% NaOH	....	....	Not present
Residue of furfurol destillation	....	....	Not present
Straw Pulp	+ +	....	Good
Sulphite Pulp, bleached	+	....	Present
In 17.5% NaOH	——	——	Not present
Purified Pulp. 94% cellulose	+	——	Present
Pine Wood ClO_2, Na_2SO_3	+	....	Good
Beech Wood Na_2SO_3	+ +	....	Good
Beech Wood extracted with 2, 5, 8 and 15% NaOH	Very weak +	....	Only slight
Esparto Pulp	+	....	Present
Bast Fibers			
Ramie, ClO_2, Na_2SO_3	Weak +	....	Weak
Flax ClO_2, Na_2SO_3	Weak +	....	Weak
Flax untreated	+	+	
Cotton, raw	....	....	Good
Cotton, wadding	....	....	
Cotton, bleached	....	....	Present
Seeds of Brazil nuts, ClO_2, Na_2SO_3	....	....	
Mannan	....	....	
Xylan	....	....	
Cellulose	....	....	
Newspaper	....	Instantly + +	

33) + + means strong violet coloration + Violet coloration. .... No coloration.

of analogy. As further sub-microscopical structural elements the rows of micelles and the micelles of Naegeli[35] have to be considered. Whether they are held together by a binding material, can not be said with certainty. Towards the lumen, the tertiary layer separates the cell wall from the plasm. Its substance is still unknown. Besides, transverse elements are built into the fiber, separately for each layer. They are tightly connected with the primary layer and the membranes of the second layer thus causing the bead-like swellings in certain reagents.

The real substance of the fibrils is cellulose. Whether xylan and mannan,[36] which were isolated in substance from pulp, are capable of forming fibrils is not known. It has been found that mannan is crystalline.[37] It is still doubtful whether xylan belongs to the structural elements of the wood fiber. Bamboo pulp, for instance, contained 19% pentosan. After the parenchyme cells had been sieved from the others and examined for their content of pentosan, the latter amounted to 39%. The peripheral layers of the cells were strongly corroded, an indication that they are containing the wood gums while the inner layers of the secondary wall contained cellulose.

The hemi-celluloses generally appear to be deposited more in the outer layers. Thus the mannans in the seeds of Brazil nuts likewise were found in the secondary membrane, and it is known that the outer wall of some bast fibers behaves differently from the inner cellulose layers towards color reactions. What the situation is in the tracheids of conifers, can not yet be stated with certainty; it can only be supposed that the hemi-celluloses here also are deposited in the peripheral sections. On the whole the topographical description of the

[35] C. Naegeli and S. Schwendener, *Das Mikroscop*, Leipzig, 1877, 2nd edit., p. 424 ff.; C. v. Naegeli, *Theorie der Gaerung*, Muenchen, 1879, p. 126.
[36] M. Lüdtke, A. 456, 201, 1927; K. Hess and M. Lüdtke, A. 466, 18, 1928, M. Lüdtke, A. 466, 27, 1928; K. Hess, M. Lüdtke and H. Rein, A. 466, 58, 1928.
[37] See here K. Hess, *Die Chemie der Zellulose und ihrer Begleiter*, Leipzig, 1928, p. 79 ff.

different chemical individuals is still rather uncertain, since the mostly used chlorzinc-iodine reaction is characteristic of several carbohydrates. But it can at any rate be determined that certain structural elements are formed by certain uniform substances.

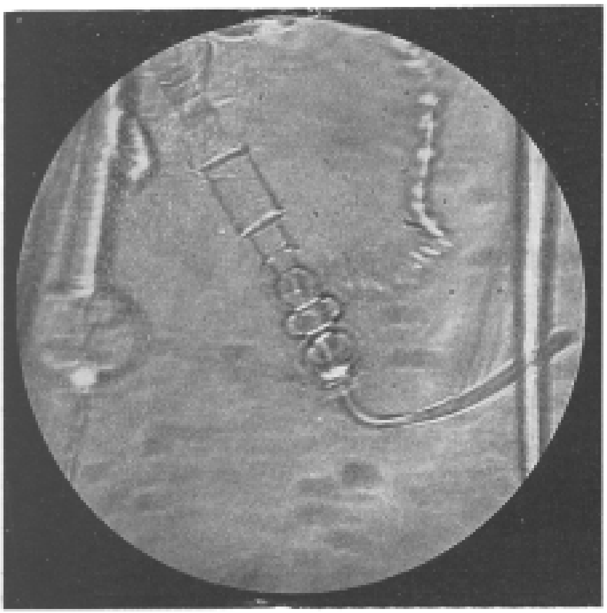


Figure 3
Bamboo Fiber X300 Magnification
Transverse elements of individual layers are in different places.

For this reason, a state where the chemical components are mixed can not be considered, much less the existence of a chemical linkage between the substances of individual structural elements. Likewise, it would be erroneous to assume lignin and cellulose to be chemically bound. The former can be extracted without producing a decomposition of fibers or cells. Besides the primary lamella separates the two substances preventing adsorption of lignin by cellulose. Furthermore, celluloses treated with esters or ether do not give the chlorzinc-iodine reaction while the vegetable membrane reacts readily provided the cellulose is freely accessible, which is not always the case, since wood, for instance, splits very easily along the middle lamella. But it can always be made accessible by destroying the middle and primary lamellæ by grinding and, of course, by chemical means.[38]

The phloroglucin-hydrochloric acid reaction has been used for determining lignin in the cell wall but the results are not beyond question. Equally unreliable is the sulphuric acid reaction because this reagent leaves undissolved not only lignin but also a substance designated as cutin.[39] As long as it is not definitely known just what lignin is and which groups have to be included in its complex, it will always remain a questionable task to undertake its qualitative, or more so its quantitative determination.[40]

Although it can thus be proven that cellulose can not form a chemical compound with lignin, there still exists such a possibility for the foreign substance in the interior of the fiber. But factors which speak against it are first the negative result of the chlorzinc-iodine reaction with all cellulose derivatives, and second the existence of cells which do not, or only in very small quantities, contain the foreign

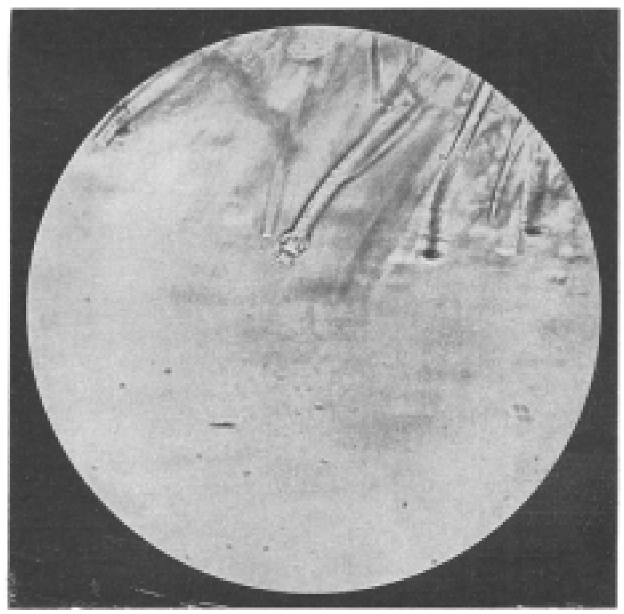


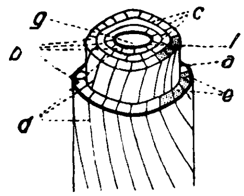
Figure 4
Fibers of Bleached Straw X200 Magnification
Layers and swelling less visible than in Figs. 1-3 on account of bleaching.

[38] See Lüdtke, B. 61, 465, 1928.

[39] M. Lüdtke, *Zur Kenntnis der pflanzlichen Zellmembran* III. A. 466, 27, 1928.

[40] See 39, The data of G. J. Ritter, *Journ. Ind. Eng. Chemical,* 17, 1194, 1925, who by means of the sulphuric acid reaction determined 75% lignin in the middle lamella and 25% in the secondary lamella.

substance but still contain cellulose. The ratio of foreign substance to cellulose, therefore, is varying greatly. For these reasons, a chemical combination appears most unlikely also in this case.



Sketch

a, Primary lamella
b, Layers of secondary lamella
c, Tangential inner skins between layers
d, Spirals
e, Fibrils or primitive fibers
f, Tertiary lamella
g, Lumen

The author has attempted to give as complete a picture as possible of the structure of the vegetable fiber, as far as it can be determined microscopically, by utilizing biological and chemical methods. The experiments for exploring the submicroscopical structure, which would follow this work, are known and it is not intended to discuss them in this connection.