ON THE ORIGIN OF LIGNIN IN THE CELL WALL

by

K. GRIFFIOEN.

TABLE OF CONTENTS.

STATEMENT OF THE PROBLEM.

> **Unfortunately, the chemistry of wood, as well as of natural organic materials in general, still represents a series of complicated problems, and, unless these are first carefully worked out, one could not expect to unravel the chemistry of decomposed wood.... (127).**

This quotation very clearly shows that we are in need of a better knowledge of the chemistry of wood, and of lignin, the characteristic substance of lignified elements. Numerous investigators have been engaged in the study of this compound and its origin and their result is analogous to that of KUERSCHNER, one of the well-known lignin-experts: „auf Grund der bisherigen, oftmals entgegengesetzt gedeuteten Untersuchungen über Ligninkörper ist man allerdings nicht in der Lage, einen genauen Ligninbegriff chemisch festzulegen (71)". These scanty results are caused by the lack of suitable methods of lignin determination and, moreover, by the very complicated and almost inextricable chemical structure of the cell wall.

We thought it important to study this problem once more and in this study we wanted to follow another way by the inclusion of a more botanical investigation into the chemical work. If we should try to investigate the process of lignification as it proceeds in the growing plant, it might be possible to trace the origin of lignin. If we should, moreover, be able to indicate a specific mother substance, present in the young cell walls, yielding lignin in the ageing process, this might be a step forward and an important starting point for further work on this subject.

After a survey of the structure and composition of the cell wall and of a number of theories concerning lignification (chapter I and II) we shall report upon the analytical results of determinations of plant constituents at successive stages of growth (chapter III) and the significance of these results, especially in relation to the origin and further fate of lignin (chapter IV) which was studied in detail (chapter V).

Apart from macrochemical analysis microscopic and microchemical studies were performed and their results compared (chapter VI). Finally, after the discussion of a number of factors affecting lignification, one of these factors — the oxidation-reduction potential of the living plant tissues — was investigated more in detail (chapter VII).

CHAPTER I.

A general Survey of the Composition of the Cell Wall.

§ 1. *The chemical composition of the cell wall.*

The vegetable cell consists of a wall and protoplasmic contents usually including one or more vacuoles. From the earliest stages of development the wall forms an important part of the cell and during the life of the cell it undergoes important alterations. During the longitudinal growth of the plant the wall consists of two layers: the middle lamella and the primary wall. The middle lamella is a layer which two adjacent cells have in common.

After the termination of the longitudinal growth much new material can be deposited against the existing wall. In this way the secondary thickening layer is formed. This layer may thicken enormously and is often built up of lamellae. In some cases a thin tertiary layer of another chemical composition may be present.

Besides this thickening all kinds of chemical transformations may occur in the wall. The lignification is one of the most important alterations of certain cell walls. During this process a product appears in the wall which gives the latter a special character. This lignification has been studied more closely in this work.

For completeness' sake the occurrence of a cuticula on the walls of the epidermis is mentioned. However, in this study the cuticula will not be considered.

The young cell wall has arisen from the so-called cell plate. This cell plate appears after the mitosis probably from the thickening part of the fibers of the nuclear spindle between the two daughter nuclei. These spindle-thickenings fuse to form a plate growing to the periphery of the mother cell and dividing the latter into two daughter cells. But the opinions about the exact origin of the cell plate are still very devided. There is also no agreement about the fact whether the wall is formed immediately from the cell plate as a whole by chemical alterations or is formed by the splitting of the cell plate. Concerning the chemical nature some investigators assume that the cell plate consists of living protoplasm, while other authors accept quite another composition in connection with an eventual transformation of this plate into the middle lamella. Seifriz (116) communicates in his survey on the cell wall: "Conjectures only can be made as to the chemical nature and the fate of the primitive wall or cell-plate. It may be an elementary form of cellulose, or it may be pectin, calcium pectate, or lignin...." and further "If the cell-plate is the progenitor of the middle lamella, then its composition is probably pectin or lignin provided the middle lamella is distinct from the primary wall and the intercellular substance which does not appear to be true."

While from the youngest stages of the wall very little is known, we know much more about the composition of the middle lamella. (N.B. in old cell walls (e.g. of wood) the true middle lamella together with its bordering primary layers are called "middle lamella"; it is better to say here "composite middle lamella".)

The middle lamella of the young cell wall consists of insoluble protopectin *) often accompanied by calcium pectate. The localization of the pectic matter in the plant has been accurately examined by Mangin (74, 75). It is not certain whether pectin is present originally in the wall or whether it only appears after cellulose. Pectin chemistry has materially advanced by the work of Von Fellenberg (33) and Ehrlich (26, 27). In this connection we refer to the clear survey of Sloep and Ripa (120), which was recently published.

It may be of importance to briefly review our knowledge of pectin chemistry in view of the radical transformations which take place in the pectinous middle lamella during lignification. The above-mentioned investigations enable us to recognize the following relations;

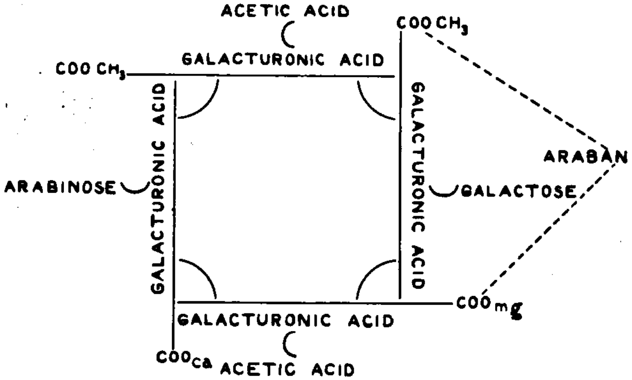
insoluble protopectin = soluble pectin + hemicelluloses
pectin = acid pectin + methanol
acid pectin = polygalacturonic acid + hemicelluloses
(+ in some cases acetic acid)
polygalacturonic acid = long chain of galacturonic acid molecules.

---

*) Protopectin is the correct name for pectic matter in the cell wall, but for convenience' sake we shall use in our study the well-known term "pectin".

In nature the various stages of this pectin breakdown are realized by the action of enzymes.

The exact constitution of pectin is not yet known. Many investigators accept a molecule of tetragalacturonic acid as the nucleus of the complex. For orientation we give a structural formula according to Ehrlich (27):



Of walls consisting of pectin no birefringence is observed. Pectin swells easily and may be extracted with dilute acids or dilute solutions of salts of organic acids. In the cell walls its presence can be demonstrated with ruthenium red. However, this dye is not specific for pectic substances. Besides in the middle lamella pectin may be present in the thickening layers of the cell wall and probably for the greater part in lamellae alternating with cellulosic lamellae (Anderson (5)). O'Dwyer (83) mentions a case of a convincing pectin reaction in the tertiary layer.

We do not know if still other products than pectin are present in the middle lamella of the young cell wall. In our investigation the middle lamella shows hardly any reaction with chlorzinciodide.

In one of the next chapters the possibility of the transition of pectin into lignin will be discussed.

The primary and especially the thick secondary wall consists for the greater part of cellulose incrusted with pectin and hemicelluloses.

It is not our intention to discuss cellulose in detail but it may be important to mention facts pertaining to the composition and formation of the cell wall (Frey-Wyssling (39), Seifriz (116)).

The constitution of cellulose is rather well-known, especially as the chemical investigations have been completed by X-ray analysis. The basic molecule is glucose with a ring structure. Two of these molecules are coupled to cellobiose with concomitant dehydratation. The cellobiose molecules are united into a large straight chain. The number of component cellobiose molecules cannot be stated with certainty but appears to be between 50 and 100. The formed cellulose molecules are joined to crystallites which build up the cell wall.

It is not sure that cellulose in its finished state enters the cell wall from the protoplasm (31) or is constructed from its supplied building blocks. According to HESS (53) the young wall does not consist of cellulose but of the so-called primary substance. The latter is amorphous and does not give an X-ray pattern. We believe that this substance may be a progenitor of cellulose consisting of a few building blocks and arranged in the wall without orientation. It may be also a carbohydrate with an amyloid structure as ZIEGENSPECK (131) accepts for the young wall.

Cellulose clearly shows a crystalline-structure and also birefringence by its typically oriented micellar construction. The presence of cellulose can be demonstrated in the wall by means of the blue colour obtained with chlorzinc-iodide.

Hemicelluloses are a vaguely circumscribed, heterogeneous group of carbohydrates (HAWLEY (50)). They are polyoses which may be divided into two groups according to the component sugar. We recognize pentosans (consisting of pentose-chains) and hexosans (originated from hexoses) and also mixed hexopentosans. Many investigators include the polyuronides which contain both sugars and uronic acids. A close relationship appears to be between hexoses, pentoses and uronic acids.

Hemicelluloses are distinguished from cellulose by their ability to be hydrolysed by dilute acids and by their solubility in dilute alkalies. In the normal cell wall they are present together with cellulose, but they may also occur as reserve substances. This is especially the case with hexosans (mannans, galactans, etc.). The most important pentosans are xylan and araban. These pentosans are partly combined with pectin.

According to KÖNIG (67) the softwoods contain a large quantity of hexosans and few pentosans, while the reverse applies to hardwoods. We should like to mention that in contradistinction to this fact BAILEY (7) found a large quantity of pentosans in the middle lamella of the cell walls of the Douglas fir. In our material, the cell walls of the sunflower stalk, only pentosans appeared to be present. Pentosans yield furfural by destillation with 12 per cent hydrochloric acid, while hexosans do not yield furfural. Therefore the determination of pentosan is performed by transferring these products into furfural and precipitating the latter as phloroglucide.

Incrusting substances such as cutin and suberin only present in certain cell walls are left out of consideration. This paper chiefly deals with lignin.

§ 2. *Lignin of the cell wall.*

Lignin appears to be a secondary substance. Except for a single vague suggestion that lignin should be present in the cell plate, it is generally accepted to be deposited afterwards in the wall. It is never found as an independent wall substance. It is already present early in the small vessels of the young seedlings (14). During the development of the plant the lignin content may increase considerably.

To the older investigators it was already known that by dissolving the cellulose from wood a residue is left. They assumed this residue to be an incrusting substance from which a part was no longer hydrolysable and only could be extracted by the aid of an oxidation process (Payen (88)). Afterwards Schulze (111) and Frémy (35) investigated its properties and the first-mentioned author employed the name "lignin" in the sense in which we use it at present. Afterwards many investigators have worked on lignin but up till now no satisfactory definition of this substance has been given (see, for example, the report of Ender (29)). We cite one of the least incomplete descriptions:

"Lignin ist ein aus Kohlenstoff, Wasserstoff und Sauerstoff bestehender Stoffkomplex und Zellwandbestandteil, der gekennzeichnet ist durch hohen Kohlenstoff- und Methoxylgehalt, leichte Oxydierbarkeit (und Halogenierbarkeit), Unhydrolysierbarkeit, Löslichkeit in Alkalilaugen und sauren Sulfiten bei hoher Temperatur und durch gewisse Farbreaktionen, von denen die mit Phenolen und Aminen auf die Anwesenheit einer Aldehydgruppe hinweisen" (Kalb (61)). Consequently lignin is a complicatedly constructed substance only preferably present in specific cell walls.

The presence of lignin in the wall may be demonstrated by means of a large number of microchemical reactions. These reactions with substances belonging to different chemical groups are mostly staining reactions and in many cases not specific for lignin itself but only specific for definite groups (e.g. aldehyde groups) in lignin or possibly only specific for other products present in traces next to lignin (CROCKER (21)). The greater part of the reactions is therefore not very convincing and criticized by many authors.

We mention the colour reactions with salts of primary and secondary aromatic amines and their derivatives (e.g. aniline sulphate), those with phenoles and phenole compounds in strong acids (the best-known and mostly-used reagent is phloroglucinol-hydrochloric acid, staining red the lignified elements), the reaction of MÄULE with potassium permanganate followed by hydrochloric acid and ammonia treatment (with remarkable differences in colour between the lignified walls of soft- and hardwoods!) and the reaction of CROSS and BEVAN with ferric-ferricyanide yielding Prussian blue. Each reaction mentioned is caused by a chemical process. On the other hand the staining of lignin with basic dyes might be considered as adsorption phenomena.

The various reactions carried out on the same material do not appear to agree. SCHINDLER (106) has investigated this point by comparing the reactions with aniline sulphate, phloroglucinol and potassium permanganate on various objects. Yet the researches of SCARTH, GIBBS and SPIER (103) are indicative of a good agreement between the reaction with phloroglucinol, the capacity of basophilic staining and the chemical examination (by means of extractives) of the quantity of lignin present. Pointing to the insufficient accuracy of the existing staining methods FRIESEN (36) worked out a new method. Black mercury sulphide is hereby precipitated on the lignified elements. His criticism of the methods used is, however, one-sided and not all together correct. We have not succeeded to apply this method on our sections of the stalk of the sunflower; the black colour of the woody elements did not appear and the whole tissue turned greyish.

Apart from the staining reactions the presence and localization of lignin may be studied to advantage by removal of the other wall substances. In this case the structure of the wall and of the entire tissue remains unimpaired. Very significant results are obtained by treatment of the sections with 72 per cent sulfuric acid. The carbohydrates are hydrolysed and the lignin remains localized. The largest amount of lignin may be found in the middle lamella which is the former pectin-layer of the young walls (97). HABERLANDT (46) has shown already that the middle lamella is strongly lignified and the investigations of **Bailey** (7) proved that this lamella may contain about 70 per cent lignin. It will appear also from our study that lignification begins in the middle lamella. In the thickening layers lignin may be present but to a much less extent. While the presence of lignin in these layers may be demonstrated by staining reactions, it appears from treatment with sulfuric acid that practically nothing is left.

If a tertiary layer is present, this layer may be lignified more markedly than the secondary layers.

Some investigators believe that in the cell wall lignin is bound to cellulose like an ester or a glucoside. But it is better to accept the free occurrence of lignin next to cellulose.

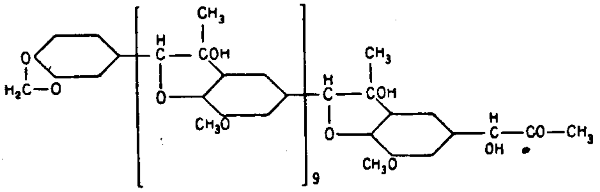
In general only specific elements show lignification. At all events the greater part of the xylem bundles and in many cases the bast fibers show lignified walls. In older plants the medullary parenchyma may lignify and under special conditions the epidermis cells too (e.g. the needles of the conifers). Cases are known of lignification in the walls of the phloem (**Boodle** (12), **Alexandrov** (2), and confirmed by us in sections of the stalks of *Helianthus*).

§ 3. *Chemistry of lignin.*

Lignin appears to be one of the most complicated substances of the wall. Till now no definite constitution is known. Apart from the very insufficient and unreliable isolation the vague definition of the concept "lignin" has served as a check to our advance. The botanical concept "lignin" possesses no chemical significance. The appearance of the lignin reactions was the sign of lignification for the botanists, while they were not interested in the chemistry of the compound. **Freudenberg** (38) objects to this state of affairs and exclaims: "Lignin ist eine durch chemische Reaktionen und Zusammensetzung gekennzeichnete chemische Substanz oder besser gesagt, ein Gemisch einander äusserst nahestehender Substanzen. In diesem und keinem anderen Sinn sollte die Bezeichnung Lignin auch bei kolloidchemischen, technischen oder botanischen Betrachtungen verwendet werden".

Lignin is a substance with a highly polymer, colloidal character, insoluble in water and in the greater part of the organic liquids. The molecule consists of a nucleus containing cyclic and aromatic nuclei bearing carboxyl, methoxyl and hydroxyl groups of which the latter ones have partly a phenolic nature. The presence of aromatic nuclei in lignin could be proved by the results obtained with sublimation, with dry destillation and with alkaline fusion yielding phenoles, phenol-carbonic acids and other benzene derivatives. The existence of the various side-chains with their different groups could be demonstrated by characteristic reactions.

Many authors have given different and speculative ideas on the constitution of lignin. We only mention those theories which are important for demonstrating the relationship of lignin to other wall- and plant substances. Afterwards this relationship will be discussed more in detail. **Schrauth** (110) accepts as lignin molecule a complex consisting of four six-atom rings and three five-atom rings; **Marcusson** (76) seems to suppose a furan ring; **Klason** (63, 65) assumes, on many grounds, a polymerisation product of compounds closely related to coniferyl aldehyde and **Fuchs** (42, 43) suggests a condensed system of six carbon rings. **Freudenberg** and co-operators (37) carried out a large number of investigations and contributed much to the knowledge of a very acceptable constitution. They concluded that the principal building stone of lignin must be dioxy-acetyl carbinol and gave as the structural scheme:



Lignin should originate from this molecule by a 20—25 fold condensation allied with ring-closure to internal ethers. They believed that the coupling of these stones is a process of dehydratation. Since the side-chains are fixed to the periphery of the central nucleus in all directions, lignin should have a tridimensional, branched structure very suitable as an incrusting substance. As a result of this structure further polymerisation to still larger molecules is very well possible.

§ 4. *Further properties of lignin and lignified walls.*

The isolated lignin will surely differ from the genuine lignin in the walls because the isolation interferes with the constitution. The isolated lignins are yellowish-brown to dark brown substances (according to the method of isolation), the X-ray diagram of which shows their amorphous and isotropic nature. They are heteropolymer and highly molecular with properties dependent upon the degree of polymerisation. (STAUDINGER (124) cannot accept these large molecules because of the very low viscosity of the solutions.) The lignins with the smallest molecules are soluble in alcohols, in a state of higher polymerisation they are still soluble in alkalies but after increasing growth of the molecules they become less and less soluble. In one of the next chapters this important fact shall be discussed. Lignins and their derivatives should give characteristic absorption bands in the ultraviolet region. Lignin substances are therefore clearly distinguishable from cellulose (123).

In the cell wall lignin occurs in the cellulosic frame without a special localization in lamellae. The wall consists of rod-like, crystalline, anisotropic cellulose particles imbedded in a lignous matrix. The combination of these two substances (small rods of cellulose alternating with lignin) forms, because the refractive index of both substances is different, a so-called "Mischkörper" of WIENER, a system with a strong birefringence in polarized light known as configuration- or bacillary-birefringence. The cellulosic rods may be dissolved out of the wall by hydrolysis with strong acids. The unimpaired, though very shrivelled lignin mass, remains without losing its cohesion (FREUDENBERG (38), FREY WYSSLING (39)).

Besides the aforesaid optical property of the lignified walls the beautiful fluorescense phenomena must be mentioned. Young wood gives a bluish-green and very old wood a greyish fluorescense, while cellulosic walls do not fluoresce at all (EICHLER (28)). On the contrary cellulosic walls stained with chlorzinc-iodide show dichroism and strongly lignified walls have lost this property.

In connection with the mechanical properties we may say that lignified elements show a very appreciable strength of compression.

VON SCHELLENBERG (104) pointed out that there should be no differences between non-lignified and lignified membranes in the degree of rigidity, ductility and swelling capacity. SONNTAG (122) seems to have found that the swelling capacity and the tensile strength of lignified walls are much smaller than of non-lignified walls. In connection with the further physical and chemical properties of lignified elements we may refer to a paper which was recently published in "Zellstoff-Faser" (135).

CHAPTER II.

Theories on the Origin of Lignin.

On the origin of lignin in the cell wall many theories have been proposed. The way in which lignin is formed still lies in the dark because our knowledge of this complicated substance is far from complete. The diverging pictures of the structure of lignin are in close relationship both with the different suppositions in regard to the manner of its formation and of its specific building stones. We have to consider that lignification is a vital process taking place in or at the surface of the living plants elements. Therefore it is not possible to follow the whole process step by step. We are only able to isolate here and there a product on the long way of development, but as a rule we do not know which stage preceded the product obtained and by which stage it might be followed. Even we do not know whether the isolated product has quite the same structure as in the plant. In vitro the processes often ran another course as in the living plant. True lignin is only formed in the living cells and it is remarkable that the proceeding lignification is concomitant with the necrosis of the cell contents and therefore with the death of the entire cell.

We may divide the theories on the origin of lignin chiefly into two groups. On the one hand we have the theories that start from the lignin formation from soluble compounds present in the protoplasm and on the other hand those theories which accept the lignin formation from substances of the cell wall itself (if we do not accept that protoplasm occurs in the wall). In the former theories the elementary form of lignin is thought to be present outside the cell wall and in the latter cases this lignin should be present in the wall itself. Moreover, the theories stressing either the aromatic or aliphatic (heterocyclic) character of the lignin molecule play an important part in the interpretation of the facts.

In the first place we shall discuss the theories starting from building stones present in the cells as assimilates or derivatives closely allied to assimilates.

As mentioned before, KLASON (63, 65) accepts the occurrence of coniferyl alcohol in the cells. This alcohol should originate from pentoses by autoxidation (via dioxy-cinnamonic alcohol). The alcohol should be combined with sugar to a glucoside, the coniferin, entering the cambium. Here the glucoside — which CORRENS (20) assumed as in a chemical combination with the wall — may immediately be turned into a lignous substance (KÜRSCHNER (70)), or should be split again into sugar and alcohol. The liberated alcohol is oxidized to coniferyl aldehyde by oxygen present in the cambial sap. The aldehyde condenses to lignin that is present partly as a soluble reserve lignin and is partly combined with cellulose to form a wall substance. The methylation should take place by the formaldehyde accepted as the first assimilation product. KLASON has founded his theory on coniferous lignin and for the other plants he has suggested a relationship of lignin to tannins. Afterwards KLASON (64) accepts that the formation of lignin is a process of anaerobic metabolism.

CLEVE VON EULER (18) adopts the theory of KLASON and she is convinced that lignins are closely related to resins and tannins as occurring in the needles of conifers. All these compounds should be derived from coniferyl alcohol.

A difficulty for the general validity of his theory is that KLASON has founded it on lignin from softwoods. We are convinced that this lignin has another composition as that of hardwoods (we recall the differences in the microchemical reactions e.g. MÄULE). Moreover, young lignins have no or almost no methoxyl groups while these should be present according to the theory of KLASON. It is, moreover, difficult to explain the lignification phenomena occurring in those elements situated far from the cambium, especially in view of the oxygen transport. This difficulty is met in more theories on lignification.

According to ODÉN (82) the pentoses are reduced to methyltetroses whereby two of these molecules yield a derivative with a furan- and a benzene ring side by side. This product is closely allied to coniferyl alcohol, which substance is able to undergo a similar ring-closure.

JONAS (60) too accepts pentoses as building stones; these pentoses, originating from hexoses by oxidation, form furfural. The latter substance yields condensed furan rings from which lignin should originate. The furan compounds may be derived likewise from hexoses by an intramolecular dehydratation (formation of oxymethyl furfural). According to JONAS these processes are characteristic reductions.

SCHRAUTH (110), whose idea on the lignin structure we have mentioned already, assumes pentoses and hexoses both as building stones for lignin.

WISLICENUS and HEMPEL (52, 129, 130) construct a detailed theory on the formation of lignin with fructose as a mother substance. The most important idea of their theory is that the lignification should be a colloid chemical process. It is therefore called "the adsorption theory". In their opinion lignin is not a simple substance but they assume "dass das "Urlignin" im unangetasteten Holz nichts wesentlich anderes ist als *die ganze Reihe der Wandlungserzeugnisse der Fruktose* infolge von deren Enolisierung, Dehydratation, Cyklisierung, Oxydation und Kondensation und weiter durch Kettenbildung und Komolierung bis zu den hochkolloiden unlöslichen Massen, die teils adsorptiv in dem micellaren Oberflächenkörper der Cellulose-Riesenmoleküle und Membrangebilde, teils als Flockungsmassen und völlig unlöslichen Ausscheidungen auf den "verholzten" Zellmembranen und in den Intercellularräumen gespeichert werden". According to them lignin should originate from fructose in the way of an oxidation process. The highly molecular colloidal lignins should be assembled slowly in the cambial sap and be removed from the reaction-system by their adsorption on cellulose. The impulse to the lignification should be given by variations in the concentration of the saps. A current of highly concentrated sieve tube sap should pass to the cambial cells which are in a reduced state. The cambial cells should have the capacity of dividing the substances in the cambial sap into a diffusible part going to the xylem side and a colloidal part (with the lipoids) going to the phloem side. Only in the cambial cells these reactions should take place. Whereas the lignin-forming substances are still in molecular dispersion in the sap of the sieve tubes, they become highly colloidal after entering the cambium. Now it is remarkable that the substances should become more and more susceptible to oxygen after further chemical transformations. The lignin as it is present in the cambial sap is still a colourless protolignin containing no methoxyl groups but already rich in carbon (65 per cent). Afterwards the protolignin is transformed step by step into the coloured lignin under the influence of oxygen. However, the lignin-forming saps should be free of oxygen! That is why the required oxygen should be supplied by the water current from the soil through the young sapwood or from the air through the lenticells of the cortex. Oxygen should be taken up by the various intermediate products of lignin. Finally the definite lignin should be formed, which enters the interstices of the cellulose walls. Then the reduced state has disappeared again in the built-up wood. The intake in the wall should take place (as mentioned above) by adsorption of the protolignin to the cellulose. The authors concluded this on the analogy of the adsorption of the cambial saps to "Tonerdefasern".

It is still doubtful whether it is allowed to transfer to cellulose the results obtained with artificial fibers. They have not proved that cellulose has a sufficient adsorption power in regard to molecules present in the plant saps. Moreover, they have not carried out elution experiments to prove that the liberated products are lignin substances.

In relation to the concept that oxygen should be necessary for the first stage of the process of the lignification **Schwalbe** and **Neumann**'s (114, 115) work has to be mentioned. Pentoses, being necessary as building stones for coniferyl alcohol (the authors accept the view of **Klason**), should be formed by oxidation of glucose, the only assimilation product. The authors state that during the growth period the oxygen dissolved in the spring sap is not sufficient and therefore further supply of oxygen through the cortex is necessary. When the oxygen is not able to reach the cambium in this way (e.g. in consequence of oxidizable substances in the cortex) less lignin can be formed. This is also the case when the oxygen transport through the cortex is stopped artificially.

Schwalbe himself is also somewhat sceptical about this theory because he finds slight difference with or without aeration. It is possible that the free oxygen does not play an important part in the process. We suppose that oxygen passing through the cortical tissue is consumed at any case by the vital processes of the cells and never reaches the cambium in this way.

We mention here the suggestion of this author that pectic substances should be intermediate products during the formation of lignin.

We want to turn now to theories according to which other cell wall materials should play a part as intermediate products during lignification. Opinions of older investigators (**Sachs**, **Sanio** (102), etc.) shall be left out of consideration.

König and **Rump** (68) accept the formation of lignin from cellulose by chemical transformations of the molecule of cellulose (such as methylations and alkylations).

Cross and **Bevan** (22) state in their extensive work on cellulose: "Lignification is a process of continuous modification of cellulose". According to them the increase of lignin proceeds at the expense of cellulose. From many other researches, e.g. from our study, it appears that this statement is not true. We find, concomitant with an increase of lignin, also a rise of the cellulose content during the ageing of the plants.

Apart from cellulose many investigators assume hemicelluloses and especially pentosans as intermediate substances.

RASSOW and ZSCHENDERLEIN (93), considering the published data on the composition of different kinds of wood, conclude that there should be a relation between pentosan and lignin. Especially from the analyses of SCHWALBE and BECKER (113) they conclude that a low pentosan content accompanies a high lignin content and vice versa.

They say: "Diese bisher noch nicht beobachtete Tatsache der Wechselbeziehung zwischen Lignin und Pentosan lässt sich ohne Zwang durch die Annahme erklären, dass das Pentosan und das Lignin in einem inneren chemischen Zusammenhang miteinander stehen. Man muss annehmen, dass das Pentosan ein Zwischenproduct bei der Ligninbildung ist oder m.a.W. dass sich das Lignin aus dem Pentosan aufbaut". They suppose that pentosan should polymerize to lignin or should combine with other substances under formation of lignin. However, if they had calculated the data for pentosan on the total amount of dry wall substances, without counting the further cell substances, they would have found that the variations in the data disappeared. Moreover they compare hardwood with softwood.

We found, in contradistinction to the last-mentioned authors, that during ageing the increase of lignin is connected with a considerable rise of pentosan! We shall discuss this remarkable result later in this paper.

Turning to pectic substances as intermediate stages during lignification we mention that SCHWALBE (115) suggested the possibility of the formation of lignin by these substances. ODÉN (82), stressing the relationship between pentoses and pectins, accepted the latter too as an intermediate product. According to FUCHS' (43) opinion, cellulosic matter should occur first in the wall, afterwards large amounts of pectic substances and finally much lignin instead of pectin. He therefore assumes the intermediate character of the pectic substance in lignin chemistry. FUCHS' considerations on the course of this process are interesting. The formation of lignin should occur in those cells, the protoplasm of which is dying. The cells should lack oxygen but should have an abundance of water. In this case the necrobiotic respiration should proceed longest after the death of the cell and FUCHS believes "dass die Fermenten der Atmung, welche am längsten in Tätigkeit bleiben, den Kohlenhydraten der Wand Sauerstoff entziehen und ihn übertragen auf den protoplasmatischen Zellinhalt, welcher allmählich veratmet wird". FUCHS believes that the oxygen of the wall substances should be split off as hydrogen peroxide, which substance should be decomposed by enzymes. By this emission of oxygen glucalic compounds should be produced in the wall, which compounds may be converted into lignins. It should be a strong reduction process and it is very probable that pectin undergoes this reduction. The ideas of FUCHS are a good example of a theory in which the formation of lignin takes place in the wall itself. Still more investigators accepted pectin as a very important building material for lignin.

The investigation of CANDLIN and SCHRYVER (17) should be mentioned here. They state that lignified tissues contain much cellulose, hemicelluloses and lignin and only traces of pectin in contradistinction to non-lignified tissues containing, besides cellulose, very much pectin, few hemicelluloses and of course no lignin. They assume that pectic acid yields hemicelluloses by decarboxylation, the latter being converted into lignin or, as an alternative, that pectin yields lignin immediately. They performed the decarboxylation of pectic acid in vitro at room temperature by means of alkali and obtained products similar to hemicelluloses from wood.

EHRLICH (26) states that pectin may be decarboxylated in the plant by decarboxylating enzymes and in this way yields pentoses (resp. pentosans). He concludes: "Aus alledem ergibt sich die logische Folgerung, dass die einen wesentlichen Bestandteil der Hemicellulosen bildenden Pentosane der Pflanze, die Arabane und Xylane, die man früher vielfach als direkte Assimilationsprodukte betrachtet hat, mit grosser Wahrscheinlichkeit als Dissimilationsprodukte aufzufassen sind, die im Stoffwechselprozess durch Decarboxylierung aus den entsprechenden Polyuronsäuren, der d-Galakturonsäure oder d-Glykuronsäure entstehen. Die Uronsäuren selbst sind vermutlich aus den entsprechenden Hexosen durch eigenartige Oxydationsprozesse hervorgegangen, über die sich bisher nichts Bestimmtes aussagen lässt" and further: "Die Ursprungssubstanzen des Lignins sind in den Pflanzen tatsächlich die Pektinstoffe und die aus den partiell verholzten Flachsstengeln isolierten Verbindungen stellen Übergangsstufen des Pektins zum Lignin dar". EHRLICH isolated from flax pectin a "lignin" with typical properties of lignin (phloroglucinol reaction, elementary analysis, etc.).

Another good indication for the correctness of FUCHS' ideas in connection with the formation of lignin is the presence of acetyl- and methyl groups only in pectin and lignin.

BUSTON (16) is an opponent of the theories accepting pectin as precursor of lignin. He does not believe in the formation of lignin from pectin but from other carbohydrates of the wall. These precursors are related to xylans in contradistinction to pectin which is related to araban. He bases this idea on the fact that the amount of pectin is not reduced after ageing of the plants.

Studies on the lignification in a twig of a rose and the processes taking place by "starvation" and "slow drying" of cut leaves showed that the amount of pectin remained constant while on the other hand the hemicelluloses, being rather labile compounds, were converted. We do not believe that experiments with cut leaves could yield convincing results.

ZHEREBOV's (133) views are entirely different. He believes that the lignification of plant elements is not a process of accumulation of lignin but of accumulation of methoxyl groups. It is remarkable that young lignin never contains methoxyl groups. Therefore, according to ZHEREBOV, the theory of KLASON cannot be true. Furthermore, he claims that the aromatic character of isolated lignin is not present in genuine lignin but is produced by the reaction of strong acids on the lignin of the wall. The enzymatic breakdown of lignin points to a carbohydrate nature. He mentions also delignification phenomena (by fermentative action) with the removal of lignin with its methoxyl groups to other parts of the plant. From ZHEREBOV's work it does not appear what he understands by lignin itself.

It was mentioned that most of the investigators accept pentoses as primary building stones for the formation of lignin. The pentoses should be converted into lignin either immediately by all kinds of chemical reactions or via other substances of the wall. In this case it is necessary to form a sufficient quantity of pentoses by oxidation of hexoses. Now it is remarkable that free pentoses are rarely found in the plant and in this case only in small quantities. If the above-mentioned theory is true, the pentoses must be converted immediately after their origin into substances such as pectin, pentosan or lignin. But it is more probable that pentoses are rarely formed and that the formation of lignin does not take place via pentoses but immediately from pectic substances which are, properly said, oxidized hexoses.

Furthermore, it is a remarkable fact that some investigators mention the decrease of specific wall substances (cellulose and hemicellulose) during the ageing of the plant, while others observe an increase of these substances. Insufficient methods of analysis as well as variations in the material might play a part in these diverging results.

It is difficult for us to arrive at conclusions from all these contradictory data the more as these conclusions should apply to large groups of plants and not to a single species. In certain groups of plants variations in the composition and the transformation of the substances of the wall may be demonstrated but surely it is extremely unlikely that during the ageing and lignification every species follows its own way.

We shall return to this point later and compare the results obtained by chemical analysis with the microscopic observations without overlooking the physiological factors.

CHAPTER III.

Chemical Analyses of the Stalks of the Sunflower.

§ 1. *The material.*

The investigation was performed with plants of *Helianthus annuus* L. of various ages. The seeds were sown in the garden of the Laboratory for Technical Botany at Delft. The sunflower was chosen because the seeds may be bought very easily in large quantities, germinate rapidly, while the seedlings show great resistance to unfavourable circumstances. In this way plants of different ages were available during the greater part of the year and the enormous quantity of seedlings needed presented no difficulty (the seedlings contain only about 6 per cent of solid matter!). The relatively large size of the seedlings was especially advantageous in the microscopic study. Unfortunately not all seeds germinated at the same time and the growth-rate proved to be variable so that variations both in length and in diameter of the stalks of the young plants occurred. This difficulty disappeared for the greater part by employing as much material of the same size as possible. Moreover the errors were eliminated by the large amount of plants used simultaneously.

For the chemical analyses sunflower stalks of four different ages were used.

Series I. Plants of 14-18 days old. (N.B. the plants were harvested after a fortnight, allowing the slower plants a few days to "catch up".) The length of the stalks was 7-11 cm and the diameter 0.4-0.55 cm. Only the cotyledons and the first pair of leaves were present. The basal internode from the roots up to the cotyledons (hypocotyledon) and the second internode from the cotyledons up to the first pair of leaves (epicotyledon) were analysed separately.

Series II. Plants of 80 days old. The length of the stalks amounted to 50-75 cm, the diameter near the base to 0.9-1.25 cm and near the top to about 0.8 cm. The stalks were examined in four parts viz. the hypocotyledon, the epicotyledon, the 3th and 4th internode and the apical part (mostly the 5th and 6th internode).

Series III. Plants of 110 days old about to flower; nearly every plant showing a large flower bud. The length amounted to 130-170 cm, the diameter at the base 2-3 cm, the diameter at the top just under the inflorescence about 1 cm. Only non-branched stalks were used and analysed in 7 separate parts viz. the hypocotyledon, the epicotyledon, a part consisting of the 3th and 4th internodes, of the 5th and 6th internodes, of the 7th till the 9th internode, of the 10th till the 12th internode and the apical part.

N.B. In the upper part of the stalk the internodes are no longer regular since the decussate phyllotaxis is changed into a scattered leaf arrangement. The number of the internodes in a certain part of the stalk analysed may be therefore variable.

Series IV. Full-grown plants of 210 days old. The plants had already shed their seeds and the leaves were partly shrivelled, yellowed and blackened. The height was 200-300 cm and the diameter near the base 4.5-5.5 cm, the diameter just under the inflorescence 12 cm. The hypocotyledon, the epicotyledon, a part of about 10 cm taken from the middle of the stalk and the apical part of about 10 cm without the inflorescence were taken for analysis.

The plants were gathered in the morning, roots and leaves removed, and cut into various parts and dried at 35° C to about air-dry. The material was first roughly and afterwards finely ground in a desintegrator. The powder was kept in stoppered bottles. Some analyses were carried out on this material and other determinations were performed after a pretreatment of the material with ethanol-benzene.

§ 2. *The determination methods of the different plant constituents.*

a. *Determination of solid matter.*

The content of solid matter was determined by drying a weighed quantity of the fresh material in a drying-oven at 105°C to constant weight.

b. *Determination of cellulose.*

The quantity of cellulose was determined according to the method of KÜRSCHNER-HOFFER (Chem. Zt. 55, 161 (1931)) based on the fact that all substances present in the plant material are oxidized by a mixture of ethanol and nitric acid and converted into a soluble form but for the cellulose, which is not attacked and remains (unfortunately mixed with a non-soluble part of the hemicelluloses).

For this determination ethanol-benzene extracted material was used. Of this material 1 gram is taken, placed in a 250 cc flask with reflux condenser, moistened with 25 cc of a freshly prepared mixture of 20 cc 96 per cent ethanol and 5 cc concentrated nitric acid and boiled in a water bath. After an hour the contents of the flask are filtered through a filter of Jena glass. With the material obtained the extraction is repeated with the same liquid.

The remaining cellulose did not show a reaction on lignin or pentoses. After the last boiling the obtained cellulose is collected quantitatively on the filter, washed first with ethanol and afterwards with water and dried in a drying-oven at 105° C to constant weight.

Some of the samples of cellulose originating from the younger parts of the stalks have a yellowish colour in contradistinction to the white colour of the cellulose from the older parts. This colour may be due to a compound of cellulose and nitric acid present in traces. The celluloses of the older and the younger parts also differ in appearance, the former being a fibrous and loose mass and the latter a tough, leathery substance. This difference may be explained because the young parts of the stalk contain much pectic matter that lutes the cellulose fibers. The old parts do not contain much pectin, which has disappeared wholly after the treatment.

c. *Determination of lignin.*

For the determination of lignin many methods have been worked out. As the lignin content is most important for our study we shall discuss the method more in detail in one of the next chapters. We used the method of GEO A. RICHTER (Ind. Eng. Chem. 23, 131 (1931)) based on the hydrolysis of cellulose and further carbohydrates present by means of strong inorganic acids.

The determination was performed as follows:

A weighed quantity (1 gram) of the material extracted with ethanol-benzene is placed in a 400 cc beaker, mixed mith 20 cc of 72 per cent sulfuric acid and stirred thoroughly. After a 15 minutes standing period 70 cc more of the same acid is added, the mixture stirred and set aside for 18—24 hours at room temperature. It is then diluted with distilled water to a volume of about 375 cc and, after stirring, digested for 2 hours on a steam bath. The mixture is filtered through an alundum crucible (previously ignited and weighed) and thoroughly washed with hot distilled water. The crucible and contents are dried to constant weight at 105° C.

The determination could be carried out without important loss of accuracy with only half or a quarter of the quantity in small samples.

d. *Determination of the furfural-yielding components.*

In our material the following furfural-yielding substances may be expected, uronic acids (chiefly galacturonic acid which forms the greater part of the pectic matter) and pentosans occurring free in the walls or combined with pectin and eventually free pentoses. These compounds are hydrolysed by boiling with hydrochloric acid yielding furfural which can be determined quantitatively by means of the precipitate formed with a solution of phloroglucinol (method of TOLLENS, ABDERHALDENS Handb. biol. Arbeitsmethoden, Abt. 1, Teil 5, 195 (1922)).

In a 300 cc flask with round bottom provided with a separatory funnel and an outlet tube attached to a condenser one or two grams of the original, untreated material is placed and boiled with 100 cc of 12 per cent hydrochloric acid (specific gravity 1.06). The matter is distilled at a rate of 30 cc in about 10 minutes. As soon as 30 cc of distillate are collected, 30 cc of HCl are added to the distillation flask and the distillation is continued in this manner until 360 cc of the furfural containing distillates are collected *). To the total distillate a sufficient quantity of phloroglucinol dissolved in 12 per cent HCl (about 50 cc of a 0.5 per cent solution) is added. At first the liquid turns yellow which is followed by greenish black. The precipitation of the furfural phloroglucide is finished after standing overnight. The precipitate is filtered, washed with 100 cc water and dried for 4 hours in a drying-oven at 97° C.

(N.B. the precipitate was washed only with cold water since it appeared that by washing with hot water a turbid liquid passed through the funnel and the results became untrustworthy.)

The furfural phloroglucide obtained has originated from the total furfural-yielding substances including the galacturonic acid. From analyses of A. C. SLOEP (119) it appeared that about three parts of free galacturonic acid correspond to one part of furfural phloroglucide. After deduction of this amount we obtain the quantity of furfural phloroglucide yielded by the pentosans. It

---

*) The last distillate may not give a pink colour with aniline acetate paper.

appeared that in our material no free pentoses were present. The amount of pentosan corresponding to the quantity of furfural phloroglucide may be calculated by the use of the TOLLENS tables in "ABDERHALDEN'S Handbuch".

e. *Determination of pectic substances.*

Various methods for the determination of pectin have been worked out. In the first place the method of BRACONNOT (see the thesis of SLOEP (119)) was used to precipitate the pectic substances by the aid of ethanol. This method is not very exact but would give perhaps indications for the presence of pectin.

The material was extracted with a 0.5 per cent solution of citric acid for one hour at 80—85° C, filtered and the treatment repeated. The collected filtrates were evaporated to the third of their volume and diluted then with about a fourfold quantity of ethanol. The pectin was precipitated as a voluminous, gelatinous precipitate. This precipitate was washed with ethanol but we did not succeed in removing all of the citric acid. The results after drying the mass appeared to be very variable, the yields remaining far below the data obtained by the method employed afterwards. The use of lactic acid instead of citric acid did not improve the method.

Therefore we tried the method of LEFÈVRE-TOLLENS (thesis SLOEP (119)). This method is based on the liberation of carbon dioxide from uronic acids when boiled with 12 per cent hydrochloric acid. The carbon dioxide originates in our case from galacturonic acid present in pectin. The amount of galacturonic acid is a measure for the pectic substances present. The content of galacturonic acid is calculated by multiplying the obtained amount of CO_2 with the factor 4.4.

In a distillation flask with coiled-tubed condenser the material is mixed with 12 per cent HCl (100 cc HCl is sufficient for a quantity of material corresponding to 150—250 mgr galacturonic acid) and heated in a metal bath. Through the mass air is sucked which was freed of CO_2 by washing it in two bottles filled with 50 per cent K-hydroxide, a gas-absorbing bottle with soda lime and a bottle with water. During the distillation, besides CO_2, furfural is formed the greater part of which condenses in the condenser and drops again into the flask, while the remainder is absorbed in a washing bottle filled with a solution of Ag-nitrate (containing 1 per cent HNO_3 and 2 per cent Ag NO_3).

The carbon dioxide liberated is absorbed in a solution of Ba-hydroxide present in the row of bottles filled up with glass-beads to enlarge the absorption surface.

Behind these bottles an absorption-bulb with soda lime is placed to prevent the entrance of carbon dioxide.

Before the beginning of the experiment the apparatus is made CO_2-free by blowing air through it for half an hour without heating. Then the bottles with Ba-hydroxide are connected and the distillation may proceed.

It is necessary that the air passes the liquid with regularity (2 à 3 bubbles per second) and the whole distillation, which takes about 6 hours, should be carried out with great care.

The amount of CO_2 absorbed by the Ba-hydroxide solution is titrated by means of hydrochloric acid.

Most of the determinations of carbon dioxide were carried out in triplicate with satisfactory results *).

We shall briefly mention the determinations of substances which show no connection to the wall substances.

f. *Determination of ash.*

The ash content is determined by ignition of the material in a china crucible and weighing. The samples contain a large amount of inorganic matter among which much Na-nitrate is present, which nitrate decomposes at high temperature and attacks the crucibles. We changed the method in this way that we added some drops of concentrated sulfuric acid prior to incineration. The data represent therefore the ash content calculated as sulphate ash.

g. *Determination of water-soluble matter.*

The water-soluble matter was determined by boiling the samples with distilled water for 3 hours. After filtration the residue was dried and weighed. The loss in weight represents the soluble part.

h. *Determination of the content of reducing sugars.*

The determination of the reducing sugars in the water-soluble part of the material was carried out according to SCHOORL, LEHMANN and MAQUENNE (Klein's Handb. Pflanzenanalyse II, Spez. Anal. I, 786 (1932)) by means of FEHLINGS' solution.

By using the standard tables of SCHOORL the data were converted to reducing sugars, as it is not very probable that in these young plants other reducing products than sugars are present.

i. *Determination of the nitrogen content.*

Since the very young parts of the plants are still rich in protoplasm, it would be perhaps important to know the nitrogen content. It has appeared that the plants contain much nitrate in their ash, therefore it seemed indicated to separate the determination in two parts viz. the determination of the total amount of nitrogen and that of nitrogen originating from nitrate.

1. The total amount of nitrogen is determined according to the method of KJELDAHL (modification VAN DE SANDE, (Chem. Weekbl. 24, 558 (1927)). By destruction with sulfuric acid, K-sulphate and Cu-sulphate the nitrogen compounds are converted into NH_4-sulphate of which the ammonia is liberated and then titrated with sulfuric acid of known normality.

2. The determination of the nitrate content in the alcoholic extract of the material is performed according to the method of SCHLOESSING WAGNER (Klein's Handb. Pflanzenanalyse II, Spez. Anal. I, 79 (1932)). The nitrate is converted into NO by means of ferrous chloride and concentrated hydrochloric acid. The gas is determined volumometrically and compared with the volume of NO generated from a known quantity of pure Na-nitrate.

---

*) The author is much indebted to Mr. L. M. VAN DER VALK who was kind enough to carry out these analyses.

k. *Determination of the ethanol-benzene extraction.*

The extraction of the air-dry material with a mixture of 96 per cent ethanol and benzene (1 : 1) was carried out in a Soxhlett apparatus on a water bath to remove chlorophyll, fatty matters, resins and tannins which might be present. This procedure has given many difficulties. The results of the various extractions and of the duplicate determinations often disagreed too much and were, on the whole, very high. It appeared that in the obtained extracts a precipitate of crystals occurred, chiefly consisting of K-nitrate. This salt was extracted by the ethanol and crystallized after cooling.

This fact may be explained by the assumption that by the use of 96 per cent ethanol the water present in this alcohol and in the air-dry material was always sufficient to dissolve some inorganic matter. The amount of water in commercial ethanol may be slightly variable and, moreover, variations in temperature and less efficient sealing of the apparatus against water vapour play a part to cause the above-mentioned divergences. The presence of the inorganic salts is therefore very inconvenient for the exact determinations of components soluble in ethanol-benzene.

If for the sake of different determinations a pretreatment with this solution was desired, the materials were extracted with the mixture for 48 hours, a sufficiently long time for the removal of the greater part of organic matter. Thereupon the extraction was stopped, the material freed of the extraction liquid and dried.

With the material thus obtained various determinations were carried out. The data were calculated on the dry basis by determination of the part which disappeared by the extraction. This soluble matter is not mentioned in the tables (except in table 3) because of the uncertainty of the numbers obtained. Roughly estimated only 2—3 per cent of the material was soluble in ethanol and insoluble in water (chlorophyll, fats, etc.).

l. *Determination of starch.*

This determination could be omitted as only a few grains of starch occurred in the endodermis, as shown by the reaction with iodo-potassium iodide on transverse sections.

m. *Elementary analysis.*

From some preparations elementary analyses were made by Mr. L. M. VAN DER VALK.

§ 3. *General remarks concerning the determinations.*

Various determinations have been carried out according to conventional methods. The instructions should be followed carefully in order to obtain reliable results. The various methods have been employed on other materials and the question arises, whether the application of these methods on an arbitrary research material is justified and whether these methods might yield reliable results. From the literature it often appears that many investigators apply a definite method without criticism and although in many cases the wide applicability of a method seems proved, it still is an open question whether this is always the case. This question becomes of still greater importance if we want to arrive at all kinds of conclusions from the data obtained. It is very difficult, however, to control the methods. In the first place because in many cases there exists only one method which, therefore, cannot be checked and in the second place because different methods applied to the same samples do not yield the same results. The latter difficulty is a cause of many errors and will be considered further. The chemical composition of the different plant components and especially of the wall substances is often very complicated and although the methods of analysis might be exact when taken as such, the employed material may be the cause of erroneous determinations. Moreover, some wall substances could not be separated very sharply from other products and the extraction reagents left or dissolved, apart from the desired substances, also a part of these products. This case appeared to occur very often and e.g. isolated cellulose will be contaminated in most cases by the presence of hemicelluloses. Moreover, it is difficult to isolate the products in the unchanged form. In most cases the product obtained either disintegrates or polymerizes, or is oxidized or reduced during the procedure.

Now it is possible that compounds present in the plant others than the substance investigated are converted by the reagents in such a way that they become insoluble. This is the case with some plant components during the determination of lignin. Therefore the data obtained for lignin seem to be too high. However, we have to use these methods for the present as well as we can.

Still more difficulties may be mentioned and it is necessary to be very careful in drawing any conclusions from the results obtained. Fortunately this study has been carried out with a large amount of different parts of the stalks merely of the same species and, roughly taken, of the same composition. The errors made in many cases are now of the same order and influence the results in a similar way. Although these errors assert themselves less strongly in a comparative examination like this they should not be overlooked.

§ 4. *The analytical results.*

In the following tables the results of the analyses have been inserted. All data have been calculated on the dry basis and represent the average content of the parts of the sunflower stalks in question, but without calculating the moisture content of the fresh stalks. The first column shows the content of solid matter of the fresh material. *)

From every table a balance has been computed to see whether all the wall substances and the total products of the cell contents have been determined or have escaped from analysis. It appears that in nearly every case the results were very satisfactory.

Since this investigation is directed especially on one of the wall substances viz. lignin and as we want to study the connection of this substance to other wall components, a number of tables follow showing the composition of the single dry cell wall.

The data referring to pectin are given in three columns showing respectively the content of carbon dioxide yielded, the corresponding content of galacturonic acid (by multiplying with the factor 4.4) and the percentage calculated as tetragalacturonic acid which has split off 3 molecules of water out of 4 molecules of galacturonic acid. As is mentioned in the first chapter tetragalacturonic acid is the basis-molecule of pectin, probably combined with pentoses, methanol, etc. These pentoses are determined together with the free pentoses and pentosans. The methanol has not been determined, because pectin itself has not been isolated from the material. For all calculations tetragalacturonic acid has been taken as the basis for the pectic substances.

The furfural-yielding components are given in two columns; the first representing the total percentage of furfural split off from galacturonic acid, pentoses and pentosans and the second showing the amount of phloroglucide after deduction of the phloroglucide of galacturonic acid (see page 343). The tables of Tollens were used to calculate the amount of pentosan corresponding to the remaining quantity of furfural phloroglucide. The latter numbers are shown in the next column.

If we draw up the balance of the data concerning the hypocotyledons, the total amount of wall substances appears to be 52.35 per cent and the part soluble in water 48.15 per cent, being together 100.50 per cent. For the epicotyledons these data are resp. 47.60 and 48.70, with a total of 96.30 per cent. This balance is of course not very exact but still seems rather satisfactory

---

*) Since the investigation is concerned with the living plant and as water plays an important part in its economy, it would have been interesting to calculate the results on the basis of the fresh material. It is, however, a great difficulty that we obtain in this way such small numbers with no striking variations. In most parts of the stalks, except in the older, the water content is not very variable so that we shall leave it out of consideration in our determinations. Of course water plays a very important part in the chemical conversions in the protoplasm as well as in the wall.

(apparently nearly all substances have been determined). That the balance of the epicotyledons does not reach 100 per cent may be ascribed to the fact that here, besides pentosans, also some hexosans might be present, which were not analysed separately. In older stages these hexosans appeared to be absent.

TABLE 1. Chemical composition of the stalks from series I.
Age: 14—18 days
Length of the stalks: 7—10 cm (hypocotyledons 4—5 cm and epicotyledons 3—6 cm)
Diameter of the stalks: 0.4—0.55 cm
Average weight: 1.0 gram

Material	solid matter in fresh material	% calculated on the dry basis										
					pectin			furfural phloroglucide				
		ash	cellulose	lignin	CO_2 liberated	calculated as galacturonic acid	as tetragalacturonic acid	from pectin + free pentosans	only from pentosans	calculated as pentosans	water-soluble matter	reducing sugars in water extract
hypocotyledon	6.40	17.30	19.80	4.00	5.90	25.90	24.10	13.10	4.45	4.45	48.15	1.45
epicotyledon	5.75	19.70	17.45	2.80	6.20	27.25	25.35	11.10	2.00	2.00	48.70	1.65

In regard to the whole composition of the wall together with the cell contents we observe that the young epicotyledons contain less cellulose, pentosan and lignin, on the contrary more pectin than the hypocotyledons. We shall meet this fact over and over

TABLE 2. Composition of the single dry cell wall.

	hypocotyledon	epicotyledon
cellulose	37.85	36.65
pectin (polyuronic acid)	46.00	53.30
lignin	7.65	5.85
pentosan	8.50	4.20

in our further examination. The younger the walls are the more pectin and the less other substances are present.

The above table shows us the composition and the transformation of the single wall without calculating the soluble part of the cell. The cellulose content of the walls has remained nearly constant in the hypocotyledons as well as in the epicotyledon. On the contrary the walls of the former contain less pectin and more lignin than the latter. *The increase of lignin and pentosan takes place at the expense of pectin.* This conversion is apparent from the following figure (fig. 1). Afterwards we shall discuss these phenoma more in detail.

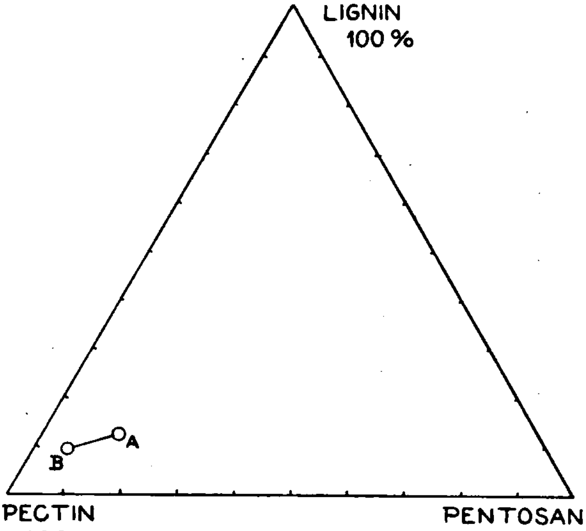


Fig. 1. **Relations between the wall substances of series I. (A = base, B = top).**

Lignin was isolated also from both parts to examine some of its properties. The two lignins showed no differences and were light brown powders contaminated with cutin from the cuticula insoluble in strong sulfuric acid. Consequently the data obtained for lignin are a little too high. Especially the lignin from the younger parts of the stalks showed these impurities, for here the very thin cuticula cannot be neglected as is the case in older stalks.

In consequence of these impurities the sublimation of the

TABLE 3. Chemical composition of the stalks from series II.
Age: 80 days
Length of the stalks: 50—75 cm
Diameter of the stalks: at the base 0.9—1.25 cm and at the top 0.8 cm
Average weight: 70 gram

Material	% calculated on the dry basis														
	solid matter in fresh material	ash	cellulose	lignin	pectin			furfural phloroglucide		calculated as pentosans	water-soluble matter	reducing sugars in water extract	ethanol-benzene extract	total nitrogen	nitrate nitrogen
					CO_2 liberated	calculated as galacturonic acid	as tetragalacturonic acid	from pectin + free pentosans	only from pentosans						
hypocotyledon	9.20	17.65	24.50	9.10	3.75	16.50	15.35	20.80	15.30	14.00	39.30	0.85	17.35	3.30	1.05
epicotyledon	6.35	17.90	22.90	7.10	5.50	24.20	22.50	19.60	11.55	10.70	40.40	1.40	18.10	—	—
3d + 4th internode	5.85	19.30	21.10	3.50	6.00	26.40	24.55	17.20	8.40	7.95	43.80	3.15	20.10	4.35	1.95
apical part	6.25	20.50	17.50	2,70	6,25	27,50	25,60	14,40	5,25	5,20	49,20	4,75	20.40	5.85	2,40

lignins hardly succeeded. The sublimates contained a large amount of fatty matter with strong birefringence and a peculiar odour. This was very probably cutin present in the lignin. Here and there groups of thin needles of vanillinic acid were visible. Both lignins were soluble in ammonia, Na-hydroxide and Na-carbonate. In their brown coloured solutions a colourless turbidity of non-soluble cutin remained visible. The lignins were insoluble in ethanol.

The chemical composition of the stalks from series II is given in table 3 (see previous page).

The balance showing the composition of the entire cell is very satisfactory, (hypocotyledon 102.25 per cent, epicotyledon 103.65 per cent, third part 100.90 per cent and apical part 100.20 per cent). For the older parts the sum amounts to more than 100 per cent. This is probably caused by the fact that in the water-soluble part a small quantity of the wall substances, e.g. pectin is present. We may conclude from this balance that practically no other hemicelluloses are present as pentosans.

From base to top we find an important increase in the percentage of pectin and a decrease in that of lignin, cellulose and pentosan, at least when calculated in per cents of the entire cells.

Besides for the wall substances regular variations in the percentages of other substances may be detected. The content of protoplasm and proteins (determined as nitrogen) is much higher at the top than at the base of the stalk. This is also the case with the water-soluble matter. The top contains more reducing sugars than the base and the increase of these sugars in the various parts of the stalk is very regular.

TABLE 4. Composition of the single dry cell wall.

	hypocotyledon	epicotyledon	3th + 4th internode	apical part
cellulose	38.90	36.20	36.95	34.30
pectin (polyuronic acid)	24.40	35.60	43.00	50.20
lignin	14.45	11.25	6.15	5.30
pentosan	22.25	16.95	13.90	10.20

In this table and in the following figure (fig. 2) is shown the composition of the single cell wall consisting chiefly of the four substances mentioned. The cellulose content of the different parts shows relatively little variation but on the contrary there seems to be a trend in the amount of pectin, lignin and pentosan. *The pectin increases enormously and lignin and pentosan decrease very much in the direction from base to top.*

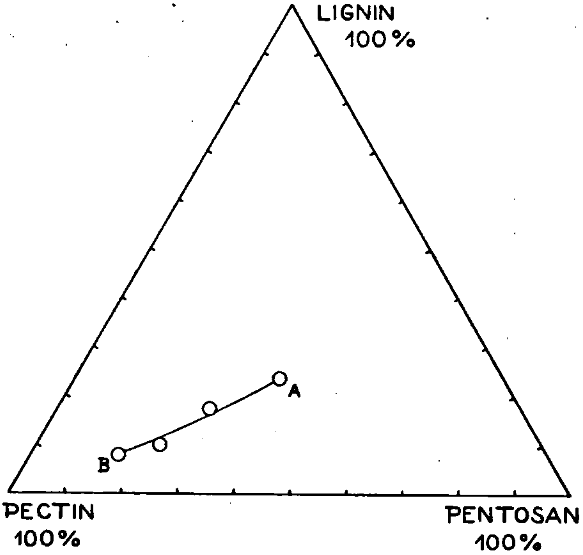


Fig. 2. Relations between the wall substances of series II. (A = base, B = top).

The lignins isolated from the investigated parts were brown powders contaminated with cutin. This was especially the case with the lignin from the top of the stalk which yielded on sublimation the fatty products mentioned above with some scattered crystals of vanillinic acid. The lignin of the hypocotyledon contained less impurities and sublimated better with the characteristic smell of vanillin.

The lignins gave with ammonia, Na-hydroxide and Na-carbonate brown coloured solutions, somewhat turbid by the cutin present and they could be precipitated again from these solutions by acid. The lignins were not soluble in ethanol.

The chemical composition of the stalks from series III is given in table 5.

The balance shows the following results: hypocotyledon 103.35 per cent, epicotyledon 103.05 per cent, 3th part 100.00 per cent, 4th part 100.40 per cent, 5th part 101.35 per cent, 6th part 99.65 per cent and apical part 100.00 per cent.

TABLE 5. Chemical composition of the stalks from series III.
Age: 110 days
Length of the stalks: 130—170 cm
Diameter of the stalks: at the base 2.0—3.0 cm and at the top 1.0 cm

	Material	% calculated on the dry basis													
		solid matter in fresh material	ash	cellulose	lignin	pectin			furfural phloroglucide		calculated as pentosans	water-soluble matter	reducing sugars in water extract	total nitrogen	nitrate nitrogen
						CO$_2$ liberated	calculated as galacturonic acid	as tetra-galacturonic acid	from pectin + free pentosans	only from pentosans					
1	hypocotyledon	11.75	13.15	34.35	16.65	2.75	12.10	11.25	25.50	21.45	19.45	21.65	2.20	2.35	0.30
2	epicotyledon	8.35	—	32.95	14.30	3.00	13.20	12.30	24.30	19.90	18.10	25.40	—	—	—
3	3d + 4th internode	6.85	22.10	28.90	9.15	3.90	17.15	15.95	22.25	16.55	15.15	30.85	2.90	—	—
4	5th + 6th „	6.65	—	26.50	6.00	4.60	20.25	18.80	20.80	14.05	12.90	36.20	—	4.30	1.50
5	7th — 9th „	6.75	23.80	23.70	5.30	4.65	20.50	19.05	19.30	12.55	11.60	41.70	5.25	—	—
6	10th — 12th „	6.60	—	22.95	4.10	4.55	20.00	18.60	18.50	11.85	11.00	43.00	—	—	—
7	apical part	7.65	22.30	17.85	4.50	4.85	21.35	19.85	15.25	8.15	7.75	50.05	7.50	6.55	1.85

The composition of the single wall substances in the different internodes is given in the next table (table 6) while in figure 3 this composition is represented graphically.

TABLE 6. Composition of the single dry cell wall.

	hypocotyledon	epicotyledon	3d part	4th part	5th part	6th part	apical part
cellulose	42.05	42.60	41.80	41.30	39.70	40.50	37.75
pectin (polyuronic acid)	13.75	15.85	23.05	29.30	31.90	32.80	39.70
lignin	20.40	18.40	13.25	9.35	8.85	7.25	9.00?
pentosan	23.80	23.15	21.90	20.05	19.55	19.45	13.55

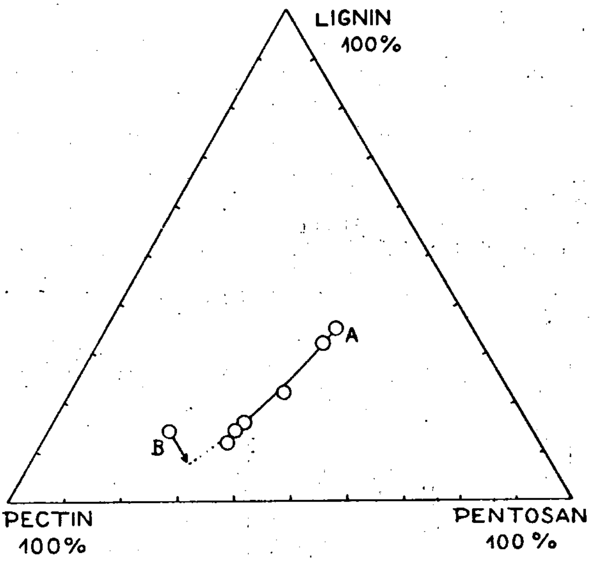


Fig. 3. Relations between the wall substances of series III. (A = base, B = top).

In the lower parts of the stalk the cellulose content of the wall is constant but in the apical part it seems a little variable. The percentages of the other wall constituents undergo changes. *In the direction of the base the walls contain less pectin and more lignin and pentosan and towards the top the percentage of pectin increases and the amount of lignin and pentosan decreases.*

In general the course of the ageing processes is similar to those in the younger stages investigated.

From various parts of the stalks from series III lignins were isolated in order to examine their properties. These lignins showed a different appearance and properties according to their origin. The lignin isolated from the hypocotyledon was a blackish-brown, amorphous powder, only partly soluble in Na-hydroxide, ammonia and Na-carbonate (it dissolved very slightly in Na-carbonate). In the sublimates of this lignin many needles of vanillinic acid and hardly any cutin were seen.

The lignin isolated from the epicotyledon showed similar properties but the solubilities were a little higher.

The lignin obtained from the middle part of the stalk was brown, soluble in the liquids mentioned above and showing by sublimation beautiful crystals of vanillinic acid; it appeared, however, to be contaminated with cutin.

The lignin from the top was coloured light brown, dissolved very easily in the alkaline liquids, but the sublimation did not succeed very well.

None of these lignins were soluble in ethanol.

TABLE 7. Composition of the full-grown stalks from series IV.
Age: 210 days
Length of the stalks: 200—300 cm
Diameter of the stalks: at the base 4.5—5.5 cm and near the top 1.5—2.0 cm

Material	solid matter in fresh material	% calculated on the dry basis										
					pectin			furfural phloroglucide				
		ash	cellulose	lignin	CO_2 liberated	calculated as galacturonic acid	as tetragalacturonic acid	from pectin + free pentosans	only from pentosans	calculated as pentosans	water-soluble matter	reducing sugars in water extract
hypocotyledon	15.00	9.90	38.85	19.30	1.95	8.65	8.05	27.10	24.20	21.85	15.90	1.75
epicotyledon	—	—	39.20	18.85	2.10	9.25	8.60	26.20	23.10	20.90	17.05	—
middle part	12.60	14.05	30.70	16.00	3 15	13.85	12.85	21.05	16.45	15.05	26.50	6.55
apical part	11.00	15.15	19.10	11.00	3.90	17.15	15.95	18.05	12.75	11.80	48.40	16.10

The balance of the wall substances together with the water-soluble matter appears to be, for the hypocotyledon 105.95 per cent, for the epicotyledon 104.60 per cent, for the middle part of the stalk 101.10 per cent and for the apical part 106.25 per cent.

Examination of the preceding table shows that in general in the full-grown plants the same trend in composition is seen as in the younger stages. There is no need to explain these data further. If we study the composition of the single wall, however, it appears that in general *the amounts concerning pectin, lignin and pentosan show the same variations as those of the younger plants,* apart from the variability of the material. (Table 8 and Figure 4 show the composition of the single wall.)

TABLE 8. Composition of the single dry cell wall.

	hypocotyledon	epicotyledon	middle part	apical part
cellulose	44.10	44.75	41.15	33.00
pectin (polyuronic acid)	9.15	9.80	17.20	27.60
lignin	21.90	21.55	21.50	19.00
pentosan	24.85	23.90	20.15	20.40

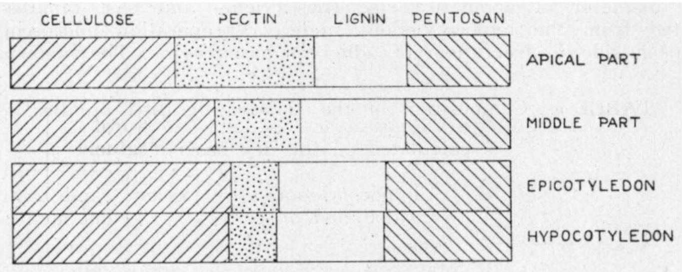


Fig. 4. Relations between the wall substances of series IV.

The divergences occurring in the full-grown stalks may be explained by the fact that the supply has stopped and that conversion of the substances has taken another direction. The percentage of cellulose, rather constant in the previous series, is here much higher at the base than at the top of the stalk. Moreover, the differences in lignin and pentosan between the young and old parts are not so marked.

The lignin isolated from the hypocotyledon was an amorphous, black powder, slightly soluble in Na-hydroxide and ammonia and insoluble in Na-carbonate and ethanol. The sublimates contained many needles of vanillinic acid and showed hardly any contamination by cutin. During the sublimations there was a marked odour of vanillin.

The lignin from the middle part of the stalk was a very dark brown product with similar properties as the lignin from the base. It was sublimated more easily and it was more readily soluble in alkaline liquids.

All the products isolated with 72 per cent sulfuric acid from the material were lignins with the characteristic properties of these aromatic substances. Afterwards we shall discuss the question if these products are real lignins or perhaps conversion-products of other components of the cells formed under the influence of the strong inorganic acid. We may mention here that our investigations have proved that the first fact is true and that the second supposition does not apply for our material.

Appendix. Analyses of lignified elements isolated from the stalk.

Since only definite elements of the stalk (chiefly xylem- and bast fiber bundles) lignify, it is interesting to investigate the lignin content of these elements in relation to that of the whole stalk. For this purpose hypocotyledons of plants of about 3 weeks old were used with a length of 5—7 cm and a diameter of about 0.5 cm. The xylem- and bast bundles were separated from the hypocotyledons under a preparation microscope and scraped outside to free them of adherent parenchyma. The material thus

TABLE 9. Composition of the isolated lignified bundles.

	% calculated on the dry basis			
	hypocotyledons calculated on:		xylem- and bast bundles calculated on:	
	total cell material	wall material	total cell material	wall material
cellulose	30.05	42.90	39.55	46.30
lignin	11.25	16.10	18.20	21.40
furfural phloroglucide (2/3 pentosan + 1/3 polyuronic acid)	16.45	23.50	26.20!	30.80!
polyuronic acid (calculated)	(18.20)	(26.00)	(± 3.50)	(± 4.00)
water-soluble matter	30.00		16.00	
total wall substances	± 70.00		85.00	

obtained was dried and ground. The yield amounted to about 400 milligrams, so that only semi-micro-determinations could be carried out of lignin, cellulose, furfural and water-soluble matter. Unfortunately there was not enough material to determine pectin. The percentage of the latter substance must be found by calculation. At first we studied the accuracy of these micro-methods performed with 50 milligrams (in duplicate) and we found that the results were similar to those of the macro-methods.

The material was analysed therefore in this way and the results compared with those of the whole hypocotyledons. Of course the results are far from beautiful since a large amount of non-lignified parenchyma is determined together with these bundles.

The remarkable differences appearing from this table should be commented upon. The xylem bundles forming the greater part of the isolated material contain a very large amount of furfural-yielding substances in comparison to the total hypocotyledon. Of course the lignin content is high, while the percentage of cellulose has undergone relatively little modification. Unfortunately too little material was obtained to perform determinations of uronic acid. It appeared from calculations that only a few per cent of pectin can be present in these bundles (we calculated the amount to 4—5 per cent). According to these data we should be inclined to assume the conversion of pectin into pentosans in the lignified bundles (high furfural content!). But the data are too unreliable, the method of isolation of the bundles too rough and in general the structure of the elements too complicated to arrive at valuable conclusions from these facts.

CHAPTER IV.

Analytical Results and their Significance in relation to the Development of the Sunflower Plant.

In the literature practically nothing is known of the chemical composition of the sunflowers. Only SHAW and WRIGHT (117) studied the composition of the whole plants at older stages of growth in connection with the contents of proteins and carbohydrates (sugars and starch). On the contrary many analytical data have been collected of various cereals of different ages. We want to discuss the paper of PHILLIPS and GOSS (90) because it turns special attention to lignin and its formation. They employed barley and analysed the stalks at 13 successive stages of growth ranging from 7 to 86 days. They analysed the wall substances as well as the nitrogenous compounds of the whole plant. It appeared that the lignin content increased from 1.48 per cent in the youngest plants to 7.74 per cent in the plants of 3 months old, calculated on the dry basis. This great increase of lignin can be noticed too from the rise of the methoxyl content of the plant. Cellulose increases from 19.0 per cent to 31.6 per cent as well as pentosan (corrected for furfural yielded by uronic acids) from 9.0 to 24.0 per cent. On the contrary the content of uronic acid decreases continuously, except for some variations. The percentage of this component is the highest in plants of about 30 days old viz. 7.82 per cent and the lowest value amounts to 4.30 per cent. Furthermore, the contents of ash and nitrogen are higher in the younger stages than in the older ones.

PHILLIPS and GOSS did not find indications that cellulose, pentosans or pentoses should be the substances from which lignin is formed. According to these authors other soluble sugars should be considered as the building stones, but they do not know whether the synthesis should take place via uronic acids (pectin) or not.

They cited in their study investigations of VERHULST, PETERSON and FRED who found an increase of the pentosan content during the ripening process of corn plants. These investigators included the determination of the furfural liberated from uronic acid. The same applies to the analyses of NORMAN who investigated the increase of the total furfural content and cellulose content (so-called CROSS and BEVAN-cellulose still containing hemicelluloses) of barley plants during growth.

The regular increase of the lignin content is mentioned by PHILLIPS from researches of BECKMANN, LIESCHE and LEHMANN (10) on rye, of PHILLIPS and co-operators on wheat and of NORMAN on barley.

Apart from these cereals only a few analyses are carried out with other plants. Various kinds of wood, however, are investigated more in detail on their chemical composition. SCHWALBE and BECKER (111) analyse the wood of *Alnus* of 9, 14 and 70 years old. They find only a few per cent of pectin, a decreasing content of pentosan (from 25.15 to 18.85 per cent) and in the heartwood an increase of cellulose from 39.6 tot 44.5 per cent and of lignin from 23.0 to 25.75 per cent.

In 1919 KÖNIG and BECKER (67) published a table of a number of hard- and softwoods they analysed on proteins, hemicelluloses, cellulose and lignin. Lignin varies from 20.69 to 29.52 per cent (the conifers contain the highest lignin values), cellulose extends from 39.97 to 49.27 per cent and pentosan from 10.80 to 25.86 per cent, the low values belonging to the softwoods and the high values to the hardwoods. In regard to our own work, we can hardly claim any relation between these various data. According to some investigators the rule: high pentosan and low lignin content in hardwoods in contradistinction to low pentosan and high lignin content in softwoods, should be a motive for the consideration that pentosans might represent intermediate products of the lignin formation. But the data in the literature do not warrant this conclusion. At first the lignins of the two taxonomic groups mentioned are not identical and in the second place the softwoods contain much hexosan in compensation of the small amount of pentosan. The existance of a relation between pentosan and hexosan should also be possible as well as a relation between pentosan and lignin. In the literature the lignin content of the softwoods is accepted as about 30 per cent and of the hardwoods as 25 per cent. As for pentosan these data are respectively 25 per cent and 18 per cent. The data are vitiated by the fact that perhaps some of the existing lignin data are too high because a part of the material may be non-lignous matter, originated by a humification and caramelisation of other plant substances by the reaction of the strong inorganic acids used for the determination. The data from the literature are therefore not very reliable and not suitable for comparison with our results. Moreover, the material employed by us is of another kind so that for this reason a comparison is also not very well possible.

If we want to study the relation between the modifications in the chemical composition and the development of the stalks, it is necessary to examine the growth of the plant.

In the summer of 1937 we carried out a large number of measurements with plants which did not yet show an epicotyledon at that time (the hypocotyledon was full-grown and 4.0—5.5 cm long) and continued these measurements until the middle of August. From that time the plants were full-grown. From the measurements it appears that at the first stage of development the length of the plants increases rather slowly after which the plants enter the great growth period with a rapid increase in length, and finally they grow very slowly. Although our measurements are performed without special precautions a rather good ROBERTSON curve results from the data as is seen in figure 5. Our results agree very well with those obtained by REED (94) for the same object.

The total longitudinal growth of the whole stalk is very complicated. As the older parts of such a stalk do no longer grow in length the proper longitudinal growth is localized in the extreme apical part. The adult parts only increase in thickness. As a rule elongation of the young internodes does not last much longer than 4 to 6 days and the further ageing of the internodes manifests itself only in the secondary thickening.

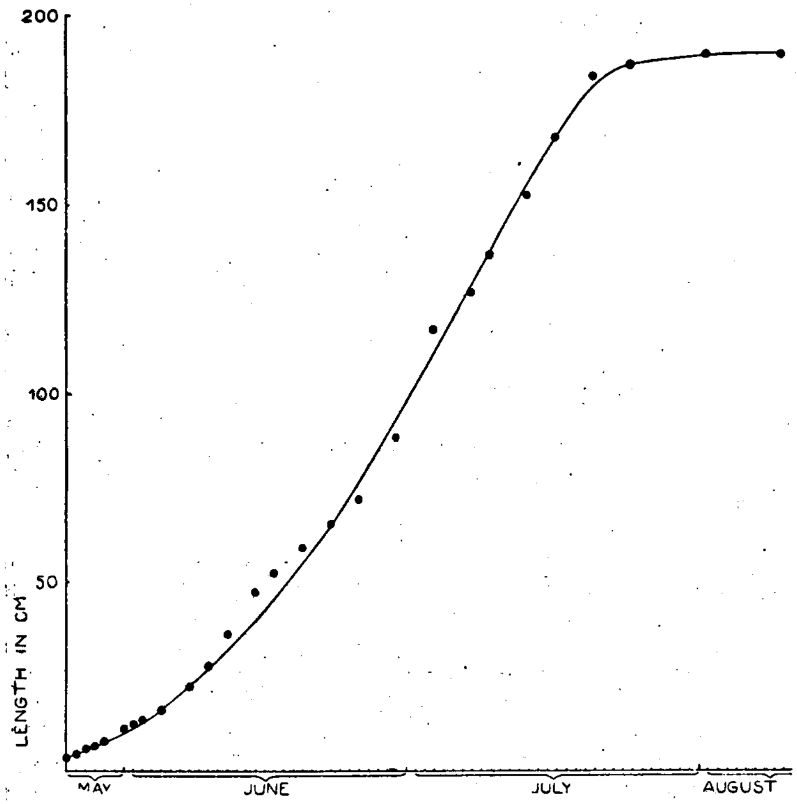


Fig. 5. Length of the sunflower plants as a function of time.

The growth in every internode is not regular along its whole length but localized at a distance of about some mm from the growing point, while the basal part of the internode does not grow any longer. It would take us too far to treat this subject at this place (see the thesis of VAN BURKOM (15)).

While the younger parts of the stalk are still growing in length, the secondary increase takes place in the internodes situated beneath them. This growth is markedly manifested in the hypocotyledon and is also distinct in the epicotyledon. These two internodes call for our special attention. The course of the secondary increase as we measured it in various stalks nearly shows the same S-curve as the total longitudinal growth.

If we want to form an idea of the relation between the modifications in the chemical composition and the development of the plant in connection with the complicated growth phenomena, various difficulties appear, which should be discussed.

Phillips and Goss (90) investigated the chemical composition of entire barley plants of different ages and plotted the percentages of the components against the age of the plants. The cereals have no typical secondary increase, so that only primary tissue occurs. They should have borne in mind, however, that in young grass stalks growth zones are found in the neighbourhood of the nodes. These zones contain meristematic tissue perhaps with another composition as the adult parts of the stalks. In *Helianthus,* as a dicotyledonous plant, the case is more complicated. While we met in the young parts only primary elements, the older parts contain products formed by the secondary increase.

We may say that only the hypocotyledon and the epicotyledon are present in the youngest stage examined. All other parts cannot be compared directly. Although already typical variations in the composition from top to base of the stalk are seen, it is not allowed to draw conclusions from this fact concerning the transformation of definite wall substances and the formation of new products. It may be accepted, however, that the development of every young internode is a recapitulation of that of the preceding older internode.

In regard to the hypocotyledon another factor of secondary importance should be considered. In the youngest stage before the unfolding of the cotyledons the building substances of the little stalk originate exclusively from the reserves accumulated in the cotyledons. Afterwards the assimilates provide the building stones for the walls. Now it is an open question whether in both cases the same substances are used in the same ratio. Of course the case of formation of building stones from reserves falls away in the internodes younger than the hypocotyledon.

We mentioned already that the hypocotyledon and the epicotyledon both are internodes present in all stages investigated. In the youngest stage the longitudinal growth of the hypocotyledon had stopped and the secondary increase had just started. All the material formed after the first stage should be considered to belong to the secondary tissue. The epicotyledon of the plants of series I was not yet full-grown. Here the longitudinal growth had proceeded for some time and was subsequently followed by the secondary increase. The further course was also comparable to that in the hypocotyledon. Because the epicotyledon still shows longitudinal growth at the youngest stage examined the analytical results are less convincing than those obtained from the hypocotyledon (see figures 7 and 8).

Besides the occurrence of the primary and secondary tissues we should turn our attention to the cells themselves and more especially to the walls. During the longitudinal growth the young walls consist only of the middle lamella and the primary layer. These walls may be stretched in the process of cell elongation. After termination of this longitudinal growth (the cells having reached their definite size) the walls begin to increase in thickness. A large amount of new material is deposited against the existing layers. The thickening layers occur therefore in the cell walls of the primary as well as in those of the secondary tissues.

Several assumptions are necessary to obtain a clearer picture of these processes:

1. During the whole vital period of the plant the chemical composition of the walls of young elongating cells remains the same.
2. During the appearance of secondary thickening layers the new substances are supplied in the same proportion as in the primary layer. During the ageing process a part of the wall components present is converted into other substances apparently more suitable for the modified requirements of the walls.
3. During the secondary growth of the stalk the cambium begins to form cells the thin walls of which show, at a young stage, the same composition as the cell walls of the primary tissue.
4. When the cell walls of the secondary tissues increase in thickness the same phenomenon occurs as is mentioned under the second point but even more pronounced.

We accept that the building substances always enter the walls in the same original proportion and, if necessary, undergo further transformations. The correctness of the first hypothesis is proved by the fact that young growing tops of the stalks of the same plant species investigated at various stages of development have mostly a rather constant chemical composition. This is also the case in our material (tables 1, 3 and 5). The microchemical reactions also give clear indications in this direction. As soon as the whole plant is full-grown the composition of the top, which does not grow any longer, undergoes considerable transformations!

Concerning the second hypothesis no convincing arguments may be brought forward. However, the course mentioned is rather probable as shall be seen afterwards in the discussion of the results. Till now no wall substances have been demonstrated in the protoplasm (except for the slight indications of Miss Farr (31) who claimed to have observed in the protoplasm very small cellulosic particles surrounded by a pectin membrane). We do not accurately know, however, in what form the substances from the protoplasm enter the cell wall.

If we accept the hypotheses mentioned above, we are able to examine the modifications in the compositions of the hypocotyledon and epicotyledon in the course of their development. We have analysed each of both internodes at four stages. After the first stage nearly only secondary tissues have been formed causing a large increase in thickness. Concomitant with this secondary increase the cell walls of the various tissues have been thickened considerably. We may accept that the increase in thickness is a measure for the age of the plant. In the following scheme this fact is illustrated (fig. 6).

The length and diameter mentioned, represent an average value of a large number of measurements.

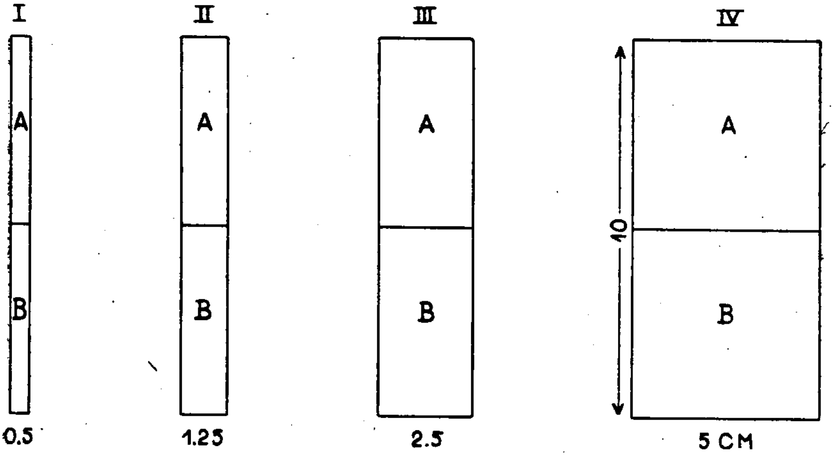


Fig. 6. The increase in thickness of the epicotyledon (A) and hypocotyledon (B) at the 4 successive stages of growth.

In the figures (fig. 7 and 8) the percentages of the wall substances, calculated on the basis of the dry wall (see tables 2, 4, 6 and 8) are plotted against the diameters of the internodes examined.

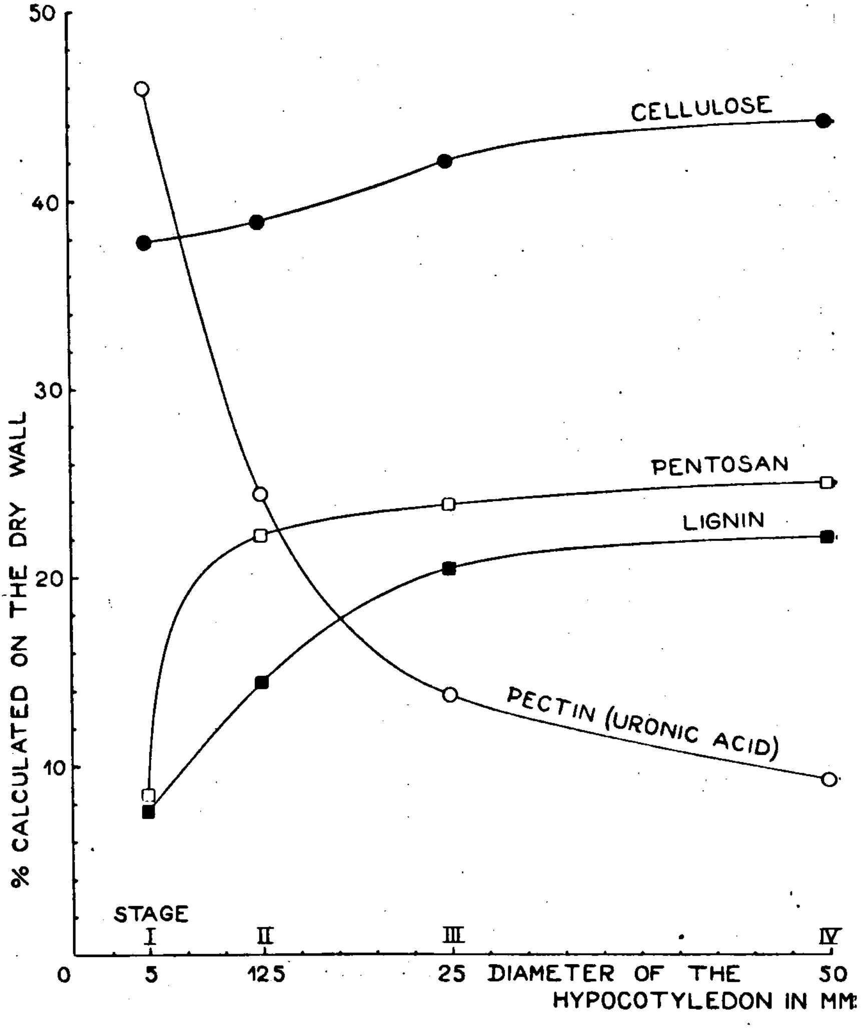


Fig. 7. Changes in the composition of the cell walls of the hypocotyledon.

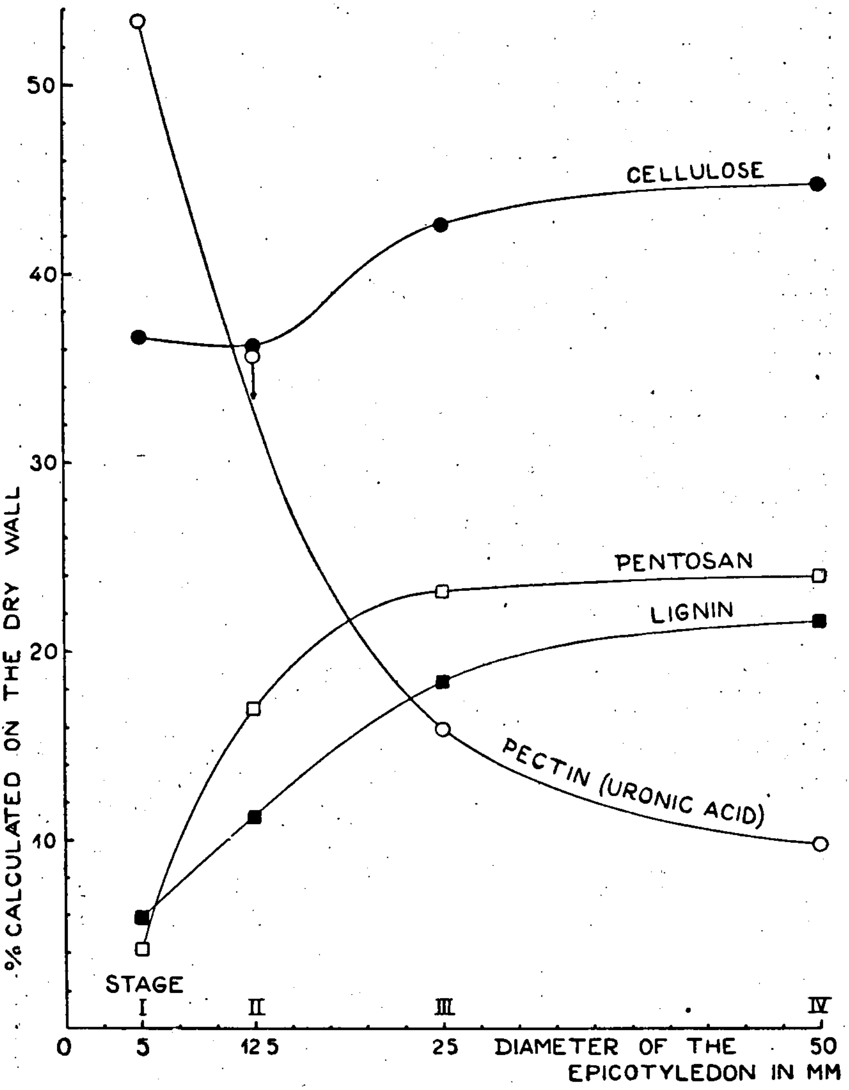


Fig. 8. Changes in the composition of the cell walls of the epicotyledon.

The cellulose content remains rather constant during the ageing of the hypocotyledon. (This is less clear in the epicotyledon and is probably caused by the fact that at the youngest examined stage this internode still showed longitudinal growth and did not contain therefore the maximum amount of cellulose.) *On the contrary the lignin content increases considerably just as, but at a lower rate, the pentosan content. The percentage of pectin (calculated as tetragalacturonic acid) diminishes rapidly, especially in the younger stages. The regularity of the pectin decrease and the lignin increase is very striking, especially after the first stage. Here it is clear that the disappearing pectin is quantitatively converted into lignin. Each per cent of lignin is supplied at the expense of a per cent of pectin.*

It is not very clear how to form an accurate idea of the transition of pectin into lignin. It is very probable that the complicated molecule as such undergoes various transformations whereby all kinds of intermediate products between pectin and lignin originate. Very strong reductions should occur to obtain lignin, for it has a carbon content of more than 60 per cent, while pectin only contains about 45 per cent of carbon. Apart from the decarboxylation of the uronic acid (from pectin) with emission of carbon dioxide, a large amount of oxygen must be withdrawn from the pectin molecule.

How this is realized we may only surmise. We mentioned already that lignification should be a necrobiotic process. The action of enzymes liberated after the death of the protoplasm will be very important in this process. When the cells are dying various abnormal processes may take place. A pressing need of oxygen will occur because the respiration processes are supposed to proceed longest of all. Free oxygen cannot be supplied to the cell in a sufficient quantity. To supply this deficiency intermolecular breakdown may take place and oxygen may be taken from pectin as the least stable and easily convertible wall substance. Pectin is converted then into products poorer in oxygen. In these products a ring-closure might take place gradually yielding aromatic lignous substances. It is not very probable that during these processes at first pectin is decomposed into simple uronic acids, with a subsequent change of these acids into pentoses which build the lignins. Free pentoses have hardly been found in the plant and this fact is in accordance with the above-mentioned theory. On the contrary pectin substances are found nearly everywhere in the plant.

Concomitant with the formation of lignin pectin may probably yield a part of the pentosans. This is shown by the trend of the curves (figures 7 and 8) and may also be derived from evidence stated in the literature. The pentosan content increases strongly in the beginning of the ageing process but later on it is more or less constant. In the older plants all converted pectin is transformed into lignin because the latter substance is necessary for the formation of large amounts of secondary wood. At the younger stages very much pectin disappears but is partly converted into lignin and partly into pentosan. This is the reason for the considerable rise of the pentosan content in these stages. Carbon dioxide is easily liberated from the uronic acids which are transformed into pentoses. In the same manner the polyuronic acids (as polymers of uronic acids) yield pentosans (as complexes of pentoses).

In vitro this process is realized by heating the acids with inorganic acid (17, 26, 34) and in the living plant it might be performed by decarboxylating enzymes. (Compare the part played by these enzymes in certain stages of the respiratory process.)

After the liberation of carbon dioxide polygalacturonic acid from pectin yields the corresponding pentosan viz. araban. Now it is very striking that in natural pectin galacturonic acid is combined with the corresponding pentose (arabinose) and the related pentosan (araban), both decomposition products of galacturonic acid (see the structural formula on page 326). In this connection we may refer to a paper of RUGE (100) who studied ageing phenomena in young membranes and supposed that pectic substances (formerly synthesized from galactose and galactan) are decomposed into pento-hexosans. It seems likely that pentosan (resp. pentose) should occur as an intermediate product between pectin and lignin. Moreover, the decomposition of uronic acid may proceed further and after the decarboxylation a dehydratation of the formed pentose may take place with the formation of furfural. Furfural might be a labile substance that could be converted easily and polymerized under ring-closure to products finally yielding lignin. We might image that these decompositions should be realized equally well in polymers of the ground-product so that from polyuronic acids pentosans originate, the latter yielding complicated furfural compounds by dehydratation. These compounds might be finally transformed into lignous substances. This procedure, however probable, we should like to amend by accepting a direct, although complicated, conversion of pectin into lignin according to an unknown mechanism.

Whatever the nature of this transformation may be, it is certain that a constant final product is not immediately formed, but all kinds of intermediate products occur which show properties of pectin as well as of lignin. The fact that the lignins isolated from younger or older parts of the stalk did not react in the same manner seems significant in this light.

The young lignins dissolve very easily in alkaline liquids (Na-hydroxide, ammonia, Na-carbonate) and the older lignins, which are polymerized more or less, become progressively insoluble in these liquids. This result is important because pectin (just as hemicelluloses) is very well soluble in alkalies. In this regard young lignins agree with pectin but during the further conversion this characteristic is lost.

During lignification of the cell walls their carbon content increases. This increase is considerable because lignin is very rich in carbon as compared with pectin and cellulose. We carried out elementary analyses in the hypocotyledons of plants at the youngest and at the oldest stage after pretreatment with ethanol-benzene solution and with hot water, so that only the wall substances remained. As a comparison the percentages of carbon and hydrogen of compounds such as cellulose, lignin, pectic acid of the flax stalk according to EHRLICH (26) and of wood*) have been mentioned.

TABLE 10. Elementary analysis of wall substances.

	% C	% H
genuine lignin (FUCHS)	63.1	5.9
cellulose (FUCHS)	44.4	6.2
pectic acid (EHRLICH)	43.0	5.7
wood (FUCHS)	50.0	6.1
cell wall of young hypocotyledons	44.2	6.5
cell wall of old hypocotyledons	47.2	6.35

Now it is probable that the carbon content of the sunflower stalk should not rise above the carbon content of wood. *The data obtained for young and old hypocotyledons demonstrated, therefore, the occurrence of a very strong reduction during*

---

*) It appeared that the wood of different kinds of trees has the same composition (FUCHS).

the ageing and lignification process of the tissues. The rise in the carbon content is caused by the enormous increase of lignin in the walls. When we accept the formation of lignin from pectin, the latter substance should liberate a large quantity of oxygen (the hydrogen content of both substances being materially alike) to arrive at lignin rich in carbon. The decarboxylation with the liberation of carbon dioxide is still very insufficient (the carbon content does not change very much when we subtract one molecule of CO_2 from a molecule galacturonic acid).

Besides this decarboxylation a very strong reduction should take place. The conditions in the living cells which are on the point of lignification must make possible the occurrence of such a reduction. In chapter VII is shown that this is really the case.

Summarizing we may say that the chemical investigation in relation to the problem of lignification has shown:

a. that pectic matter should be considered as the starting point of the lignin formation,

b. that hemicelluloses (pentosans) may play perhaps an intermediary part. Pectic matter is easily transformed into pentosan but a direct transition of pentosan into lignin seems improbable, and

c. that the lignification has to be considered as a very strong reduction process.

CHAPTER V.

The Problem of the Isolation and Quantitative Determination of Lignin.

§ 1. *Review of the literature.*

"One cause of the comparatively slow progress in lignin chemistry is the difficulty of obtaining lignin free from other plant tissues and unchanged in the process of isolation. Practically every process that has been advanced is open to objection". This statement of SHERRARD and HARRIS (118) and perhaps of a greater part of other investigators clearly demonstrates the necessity of discussing our method of isolation and determination of lignin by means of 72 per cent sulfuric acid. It is the intention to separate, by this method, a possibly uncontaminated lignin from the material without any change of the original structure. Now it has appeared that lignins, after complete decomposition of the other products, have undergone important modifications, while on the other hand a very carefully isolated lignin contains many impurities caused by insufficient hydrolysis of the other constituents.

There are chiefly two methods of lignin determination viz. a direct and an indirect method. In direct analysis all substances other than lignin are removed and indirect analysis requires the removal of lignin. Besides the pure chemical isolation methods also a biological procedure may be applied (WEHMER (128), FALCK (32), etc.). Various micro-organisms are able to decompose carbohydrates and other wall products, while leaving the lignin unchanged. It is certain, however, that in this way a pure product cannot be obtained, for on the one hand the microbes will not attack quantitatively all components and on the other hand the obtained lignin might be more or less decomposed during the complicated microbiological processes.

As common solutions used in direct analysis we mention strong mineral acids, such as sulfuric acid, super-saturated hydrochloric acid, hydrochloric acid plus phosphoric acid, hydrofluoric acid, and further alternating treatment with mineral acid and cuprammonium solution. Moreover lignin may be isolated by treatment of the material with alkalies, organic solutions (alcohols, phenols, aliphatic acids, etc.) whether or not combined with mineral acid. But this second series of methods as well as the technical procedure with sulfurous acid and alkaline bisulphites is not suitable for quantitative determination. Sometimes lignin is approximately estimated as that part of the material soluble in 2 per cent Na-hydroxide and non-hydrolysable with 2 per cent sulfuric acid (BERTRAND and BROOKS (11)). In this case the data obtained agree very well with those obtained by acid treatment.

Besides these macro-methods micro-determinations were carried out by some investigators. We only mention KLATT (66) who employed hydrofluoric acid and BAILEY (8) using formaldehyde and sulfuric acid.

It is remarkable that with the application of various methods on the same material the obtained yields may diverge markedly while, moreover, the properties of the isolated lignin may be rather variable. This can be explained by the fact that some of the non-lignous components may be not completely hydrolysed, but it also remains an open question whether more lignins, which may be isolated only by means of definite reagents, should be present in the material. A study of PAULY and co-operators (87) points in this direction, as the authors seem to have isolated from winter-rye straw seven lignins possessing different properties.

Other examples are stated by BECKMANN and co-operators (10) who isolated different lignin fractions by treatment with Na-hydroxide at various temperatures and higher pressures (1-9 atmospheres), and by PHILLIPS and GOSS (89) who extracted from straw two different lignins viz. one with alcoholic and one with aqueous alkali. It is, however, very probable that we do not deal in this case with different lignins, but with successive states of polymerisation or depolymerisation of one and the same substance.

It is very difficult to test the purity of the obtained lignins. The empirical composition is not constant but has an average of 63 per cent carbon and 6 per cent hydrogen. The methoxyl content should be another important characteristic, and the higher this content the purer the lignin should be (RASSOW and GABRIEL (92)). FRIEDRICH (40, 41) states: "Das Auftreten von Methoxylgruppen in Pflanzenteilen ist charakteristisch für die beginnende Verholzung der Zellmembran (Ligninbildung)". However, the methoxyl content of the lignins varies widely. Moreover, RITTER (98) demonstrated the presence of many methoxyl groups in his holocellulose (the total carbohydrate portion of wood) and HILPERT (54) believed to have found that these groups are not bound to lignin at all but to carbohydrates which precipitate simultanuously with the lignin. Without accepting at once the exactness of these investigations, it is in consequence of these circumstances very difficult to estimate the degree of purity of lignin.

Sulfuric acid is one of the most common reagents for the lignin isolation and determination. The concentration of the used acid is very important and varies from 64-72 per cent according to different investigators (FRÉMY (35), KLASON (62), OST and WILKENING (85), KÖNIG (68), RICHTER (96), SHERRARD and HARRIS (49, 118)). We employed the method according to RICHTER which gave the best results in the Laboratory for Technical Botany at Delft. Apart from the concentration of the acid the reaction time and the reaction temperature appeared to be of essential importance, just as in the pretreatment of the material and the working-method during and after the procedure (RITTER (99)). PALOHEIMO (86) pointed to the great influence of these factors upon the lignin yield. Thereby he obtained striking results e.g. that finally in rye straw a lignin content is found of 5.7 per cent in contradistinction to the data found formerly (about 20 per cent)! Moreover, a third part of this content should still be proteins! The disturbing influence of proteins on lignin determination was investigated by **Norman** (81) who detected that proteins present in the plant materials give insoluble compounds with lignins under the influence of the strong mineral acid (consequently a quantity of nitrogen is nearly always found in the elementary analysis of lignin!).

. Together with the proteins the carbohydrates present in the material may play a great part. **Norman** (81) states, in a comprehensive study on this subject, that after treatment with sulfuric acid the "lignin" content is apparently increased by the presence of carbohydrates. He finds that sucrose, fructose, pure xylan and crude hemicelluloses, extra added to material with a definite lignin content, cause an increase of the lignin value. Other sugars and pectic substances are without any influence. The reaction time plays an important part in this phenomenon. **Norman** already notices a great apparent increase of the "lignin" content after a reaction of the acid during 2 hours when hemicelluloses are added. **Ritter** (99) finds the same and he hydrolyses therefore previously the hemicelluloses in his material with dilute acid. **Norman** raises the question if this pretreatment may be allowed, since lignin might be attacked by dilute sulfuric acid, which seems actually to be the case (**Cohen** and **Harris** (19)). (Nowadays this pretreatment is performed, however, in many cases.) The presence of hemicelluloses is, therefore, a source of great errors!

According to **Hilpert** and co-workers (55) the sugars present in the material should play an extremely important part. Formerly these authors found that sugars yielded insoluble lignin-like substances by the action of strong sulfuric acid (fructose, xylose and xylan should be compounds very sensitive to strong acids; they yield resp. 25, 36 and 33 per cent "lignin"). The sensitivity decreased at reduction of the reaction temperature. At 6° C fructose did not yield an insoluble compound with acid. Elementary analyses of these "sugar lignins" agreed with those of lignin from coniferous wood. When **Hilpert** treated straw with sulfuric acid at 20-30° C it yielded a large "lignin" residue. He accepted in lignified plant elements the presence of fructose together with the wide-spread xylose, and also methylated sugars responsible for the methoxyl content of the "lignin". (**Norman** too seems to assume the presence of large amounts of pentoses in the plants.) When **Hilpert** treated straw at 6° C, at which temperature the sugars should be insensitive to sulfuric acid (no formation of an insoluble reaction product!), he still obtained 18 per cent of "lignin". He solved this difficulty by accepting, in straw, the presence of carbohydrates still more sensitive than the above-mentioned sugars, so that the temperature should be reduced still further to come below the limit of sensitivity. That is why he carried out the reaction at — 10° C with the surprising result that the whole material dissolved except for a small amount of inorganic salts! The formation of a reaction product was entirely suppressed! Moreover this experiment should indicate (according to HILPERT!) the complete absence of true lignin! The "straw-lignin" obtained at higher temperatures was identical with the "sugar-lignin" mentioned above. Consequently these "lignins" should have a carbohydrate nature (56). The same was stated by him for the lignins isolated from beech wood and other materials (57). These lignins should be reaction products of sulfuric acid on cellulose components*).

Now HILPERT concludes that not only the high lignin values found by the investigators are only apparent (because a great part of them are reaction products of the carbohydrates by the action of sulfuric acid), but also that even in many materials lignin does not occur at all, the isolated products being only reaction products! If any lignin should exist, it has no aromatic structure but a carbohydrate nature. An interesting example in which the view of HILPERT is surely right can be found in a paper of SCHMIDT-NIELSEN (108, 109) who met in various seaweeds a considerable percentage of "lignin" (determined with the strong acid method!) and this is hardly possible.

It is self-evident that the study of HILPERT was vehemently criticized since true lignins have been repeatedly isolated the aromatic nature of which is clearly proved by chemical analyses. SCHWALBE (112) published some of his objections in a detailed paper, demonstrating the occurrence of true, aromatic lignins after various procedures. Of course it is possible that the whole poduct or only parts of it contain an aromatic nucleus. He mentions that KLASON in contradistinction to HILPERT found

---

*) HAWLEY and HARRIS (51) prepared "synthetic lignins" from cellulose of coniferous wood by heating the cellulose at 135° C for several days. Finally they obtained a yield of 70 per cent "lignin" resembling in its properties lignin obtained from coniferous wood itself (only it contained no methoxyl!). The determination of this "lignin", however, was carried out by the H_2SO_4-method, and is, consequently, unreliable. The cellulose, perhaps partly decomposed by the heating, may be easily decomposed by acids under formation of insoluble humin-like matter.

the resistence of various sugars, such as xylose and others to the presence of concentrated acids. Kürschner (71, 72) obtained an important residue from straw at very low temperatures (also in contradistinction to Hilpert). Schwalbe explains the total solubility found by Hilpert by accepting that young straw lignin should be soluble in sulfuric acid in contradistinction to the older lignins from wood. In contradiction with his own view Hilpert should have found in beech wood 10-15 per cent lignin at 10° C. Although Hilpert assumes here the presence of still more sensitive carbohydrates, Schwalbe believes that we meet with true lignins here. Moreover, the presence of fructose and methylated sugars has never been demonstrated in straw! In this way Schwalbe adduces various arguments against the theory of Hilpert.

Also Waksman and Cordon (126) objected to his theory. They studied the lignin isolation with strong acids at different temperatures with and without pretreatment by dilute acid. At low temperatures (2° C) the hydrolysis of straw appeared to be incomplete; when the residue was treated once more with the acid at room temperature. Waksman and Cordon finally obtained a yield equal to that obtained by a direct treatment at room temperature. Furthermore, they found that cold alkali extracted less lignin than hot, but the total amount of lignin extracted by cold alkali added to the quantity of lignin left in the residue was practically equal to the percentage of lignin obtained by direct acid treatment. The same agreement was found for various other properties of lignin.

Consequently they conclude that the Hilpert hypothesis has no foundation in as far as it claims that lignin as such does not exist in the plant. Of course it cannot be denied that the chemical nature of the lignin is changed by the action of the acid (see also Schmidt (107)).

We may even raise still more objections against the Hilpert theory. For instance, it cannot explain the lignins obtained by means of procedures without acids. We already mentioned above an alkali-lignin prepared by Waksman and recall the products obtained by exclusive treatment with alcohol.

When in lignified materials no lignin should be present but only components of carbohydrate nature, how may we explain the strong increase of the carbon content during lignification? What is the ultimate nature of the lignin-like subtances yielded in large amounts during microbiological processes of wood decomposition? We surely cannot accept that the microbes affect carbohydrates in the same way as strong mineral acids, in other words we have to accept in these cases true lignin or related products.

If we consider the results given in the literature and combined with those obtained from our own material (§ 2 of this chapter), we have good reason to accept the existence of true lignins with an aromatic character. Many of the investigations of HILPERT seem, however, correct so that a revision of the lignin data given seems useful.

§ 2. *Own investigations.*

We studied more closely one of the analysed plant series (series III) to investigate whether the lignin isolated from our materials might perhaps be considered, wholly or in part, as reaction products in the sense of HILPERT.

The lignin data given in table 5 were obtained by extraction of the material with ethanol-benzene only. The carbohydrates were still present and might have been the cause of the formation of insoluble products. At the first place we removed the water-soluble carbohydrates (sugars) by extraction of the material with boiling water. In the dried, extracted material determinations of lignin, cellulose and furfural were carried out. The results are mentioned in table 11, after being calculated on the original dry basis. Furthermore the material extracted with water was hydrolysed with 1 per cent sulfuric acid. A large quantity of matter was removed, for the pectic substances dissolved and the hemicelluloses were hydrolysed

TABLE 11. *Helianthus* from series III (see table 5).

% calculated on the dry original basis									
	lignin			cellulose			furfural phloroglucide		
	A	B	C	A	B	C	A	B	C
hypocotyledon	16.60	15.00	15.15	34.35	34.20	31.60	25.50	24.35	8.60
third part	9.15	11.20	9.75	28.90	30.40	27.00	22.25	19.65	6.50
fifth part	6.00	7.70	6.75	23.70	24.30	23.15	19.30	15.10	6.00
apical part	4.50	5.20	4.30	17.85	18.50	17.00	15.25	10.20	4.50

A = original material.
B = this material after extraction with ethanol-benzene and after extraction with hot water during 3 hours.
C = preceding material after hydrolysis with sulfuric acid during 4 hours.

for the greater part. Only slightly hydrolysable hemicelluloses remained. After filtration the residue was dried and the loss in weight estimated. This residue only contained lignin, cellulose and remaining hemicelluloses determined again in the usual way. The data are given in the same table, calculated on the dry basis.

In the following table (table 12) we give the quantity of material removed by the extractions. Column 1 represents the lost quantity after water extraction (these data include the percentage of material extracted by ethanol-benzene). Column 2 shows the lost part of this material after hydrolysis with acid. In column 3 the total loss of material and in column 4 the remaining quantity have been given.

TABLE 12.

	% calculated on the dry original basis			
	disappeared after water extraction	disappeared after H_2SO_4 hydrolysis	disappeared in total	residue
hypocotyledon	25.0	29.1	46.7	53.3
third part	33.0	34.5	56.1	43.9
fifth part	43.0	36.6	63.8	36.2
apical part	52.0	44.2	73.2	26.8

The results seem conclusive. It appears that the lignin content of each of the four parts examined has remained constant within very close boundaries after water- as well as after acid treatment. *The water-soluble carbohydrates (sugars) did not yield "lignin" nor did the hydrolysable hemicelluloses and uronic acids.* The cellulose content has remained rather constant. The data obtained after hydrolysis turned out a little too low. This may be caused by a beginning decomposition of cellulose.

The columns in which the quantities of furfural phloroglucide have been inserted clearly show that a very strong decrease of furfural-yielding substances is seen after the successive treatments. This decrease, however, is of no influence upon the "lignin" content, in contradistinction with the results of NORMAN and HILPERT. Closer inspection of the data of the furfural phloroglucide shows that, after the water extraction, the content of furfural-yielding components in the hypocotyledon has not diminished in a marked way. This is certainly the case in the higher internodes of the stalk and the more we reach the top the greater the decrease appears to be. During the water extraction the following compounds may be removed: pentoses, a part of the pectin and eventually water-soluble hemicelluloses. These substances should therefore be found again in the aqueous extract. Now we have succeeded in determining this lost amount nearly quantitatively with the aid of the TOLLENS' method. To see which of the above-mentioned products may be present in the aqueous extract, we partly evaporated this extract and added an excess of ethanol. A gelatinous pectic mass precipitated. This mass was filtered off and the TOLLENS' destillation performed once more in the clear filtrate. Phloroglucinol yielded no precipitate in the collected destillates. From this it appeared that furfural was absent, in other words, no pentoses or water-soluble hemicelluloses were present in the filtrate after the removal of pectin. The furfural from the original extract originated from pectin (better said from the galacturonic acid of this pectin).

As in none of the investigated materials of serie III pentoses were present, we may suppose that also in the other growth stages no free pentoses occur in analysable amounts. This fact is very important in relation to some of the theories on lignin formation discussed in the second chapter.

It was seen that the decrease of furfural-yielding substances after water extraction was caused by the solubility of a part of the pectin in water and this seems to be greatest at the top. The various quantities of furfural phloroglucide are given only for the top and for the hypocotyledon.

The furfural phloroglucide content of the material from the top before water extraction is 15.25 per cent, after this extraction 10.20 per cent, the difference is 5.05 per cent; the percentage in the aqueous extract before precipitation with ethanol is 4.00 per cent and after treatment with ethanol 0.00 per cent. For the hypocotyledon these 5 data are resp. 25.50, 24.35, 1.15, 1.75 and 0.00 per cent.

These results are very satisfactory, considering the errors due to the conventional determinations. During the hydrolysis with sulfuric acid the most important decrease of furfural-yielding components occurs*). This is caused by the removal of the greater part of the hemicelluloses and of the pectic substances. Only the so-called "slightly hydrolysable" hemicelluloses and perhaps a few per cent of pectin are left. It might

---

*) The hydrolysis of the material must not be continued too long as finally cellulose and lignin are decomposed too by sulfuric acid.

be possible that these substances should cause the formation of "lignin". But from the obtained data it appears that probably this cannot be true. The percentage of the total residue of the material after extraction with water and dilute acid appears to be equal to the sum of the percentages of lignin, cellulose and remaining hemicelluloses (calculated as pentosan) determined in that residue. We can read this from the tables 11 and 12.

This sum amounts for the hypocotyledon to 54.95 per cent and the total residue is 53.25 per cent calculated on the dry basis. These data are resp. 43.15 and 43.90 per cent for the third part; 35.60 and 35.15 per cent for the fifth part and 25.80 and 26.80 per cent for the apical part.

If lignin should not have been present as such in the plant material but has arisen from hemicelluloses as an insoluble reaction product of concentrated acid, then the above-mentioned residue should have composed only of cellulose and hemicelluloses. It should have been smaller for the amount of "lignin" than the sum of lignin, cellulose and hemicellulose as is given above. From the preceding data this does not appear to be the case and we may conclude from this that, at all events, lignin as such must be present in the stalks of the sunflower.

According to our investigations the theory of HILPERT does not apply to our materials. We do not want to deny the correctness of many of his experiments. But our experiments surely indicate the presence of true lignin in the plant and also the reliability of our lignin data, although we know that these lignins are not entirely pure.

CHAPTER 6.

Microscopic and Microchemical Investigations of Cell Walls of the Sunflower Stalks.

§ 1. *Microscopic examination.*

A detailed description of the anatomy of the sunflower stalk is not given in this paper. We may refer to studies of JEFFREY (59), ALEXANDROV and ALEXANDROVA (2) and to the thesis of DUNCKER (25). These authors examined sections of the stalks at various heights and at different growth stages and studied the nature and development of the vascular bundles and its cambium activity. The discrepancy in their results will not be discussed here. This paper deals only with the changes of the lignifying elements.

Stalks of the same plant series as used for analysis served as material. The stalks (being respectively 17, 80, 110 and 210 days old) were gathered at the same time as those destinated for analysis and preserved in alcohol. Apart from this material also fresh plants almost of the same age were used. We examined transverse and longitudinal sections in concomitance with maceration preparations.

It was possible to prepare microtome-sections only of the young part of the stalk; from the older, lignified parts hand-sections were prepared. From the stalks of series I we made transverse microtome-sections at various levels at a distance of about 1 cm beginning just above the roots. The sections were partly from the hypocotyledon, partly from the epicotyledon and from the node between both parts. The pictures were compared with those obtained from fresh material. Besides the transverse sections also longitudinal sections were made. They were compared with maceration preparations of the material. The maceration was carried out very carefully in order not to disturb the position of the elements.

Parts of the stalks were heated for some minutes in a very dilute solution of hydrochloric acid (0.5 per cent). During this procedure the pectic substances are dissolved and the elements of the stalks could be separated by means of a pair of dissection needles. The bast fiber bundles and xylem bundles could be isolated in this way as a whole. They were washed thoroughly to remove the hydrochloric acid, placed in various reagents and examined.

From series II alcohol- and living material was used. The younger parts could still be cut by the microtome and the older ones by hand. Transverse sections were prepared at various parts of the hypocotyledon, of the epicotyledon and of the higher internodes. The sections were always prepared between the nodes unless indicated otherwise. From this series longitudinal sections and maceration preparations were also prepared.

From series III only hand-sections were made from base to top about every 20 cm. Moreover longitudinal sections were examined to follow the course of the bundles.

Finally sections were prepared from series IV at the base of the hypocotyledon, at the middle of the epicotyledon, at about the middle of the stalk and close to the inflorescence where the stalk was thinnest.

As reagents for the microscopic examination were chiefly used: phloroglucinol—HCl, aniline sulphate and chlorzinc-iodide for the demonstration of lignin, chlorzinc-iodide for cellulose and ruthenium red for pectic substances. Moreover, other sections were stained with oxamin blue, a meta-chromatic dye.

The microscopic examination was performed to study the process of lignification in the cell walls during ageing of the plants. It will be seen that the whole change of the anatomical picture is just the same as we know from classical investigations, but we believed it to be instructive to perform this examination once more for the elucidation of various lignification problems. The anatomical pictures of younger, older and adult parts of the stalks will be different in general. In younger internodes only primary tissues occur. Moreover, the primary elements will have obtained another chemical composition in the adult parts as in the young, growing internodes.

The hypocotyledon from series I is already full-grown. The next stages of this internode (from series II) will be characterized by an increase in thickness without elongation. All elements newly formed, originate from the cambium so that the original primary elements, the number of which is no longer increasing, become less conspicuous. The composition of the young growing top is practically the same in every stage. In the adult stage, however, typical modifications occur in these primary tissues which are now also subjected to the ageing process. We shall successively discuss the bast fibers, the wood vessels and the wood parenchyma in their course of development.

a. The bast fibers.

The bast bundles are composed of prosenchymatous elements. During the ageing process of these cells their walls increase in thickness and begin to lignify.

In sections made at various heights of the stalk the bast bundles are seen of course at different stages of development and lignification. The development of the bast bundles is important only in those parts of the stalks where they can participate in the mechanical system.

If we examine transverse sections from the top of the young stalk, bast bundles are already observed as groups of differentiated, very thin-walled tissue situated like caps on the outer periphery of the phloem. The cells of this tissue are much smaller than those of the surrounding cortical parenchyma. The thin walls do not give a lignin reaction but a distinct blue staining with chlorzinc-iodide (cellulose) together with an intensive red staining with ruthenium red (pectin), The walls are very rich in pectin *), especially present in the middle lamellae. These lamellae are able to swell considerably with various reagents (Zn-chloride, hydrochloric acid, alkalies, etc.). If, for instance, sections are treated with chlorzinc-iodide to demonstrate cellulose, the middle lamellae swell by the action of chlorzinc but hardly give any colour reaction. Against both sides of these swollen lamellae a thin cellulosic layer (the primary wall **) is situated, stained blue by chlorzinc-iodide. If we wash the sections treated like this and place them in a solution of ruthenium red, the swollen layers are stained dark red, indicating the presence of pectin. Especially in the younger stages this nature of the bast fibers remains the same till rather far from the top of the stalk. In the lower parts of the stalk the cell walls are continuously thickening by deposition of new wall material, while at the same time the swelling power is diminishing more and more. In the neighbourhood of the base of the young stalk lignin may be demonstrated in the middle lamellae. At first these layers are faintly stained by phloroglucinol, but the intensity of the colouration increases continuously. The middle lamellae lignify rapidly, while the thickening layers do not yet contain lignin at the first stages of this process. During ageing of the bast fibers their walls are thickened enormously and they strongly lignify at the same time. This stage has not been reached, however, in the hypocotyledon of the plants from series I. In this series the thickening layers of the fiber walls have been increased considerably but are slightly lignified (faint red with phloroglucinol-HCl and green with chlorzinc-iodide). If we compare these hypocotyledons with those from series II, it appears that these internodes have no longer elongated but have only grown in thickness. The bast bundles consist of very thick-walled cells at this stage. The thickening layers are lignified rather well and stained yellow by chlorzinc-iodide. In some cases the inner wall layer is not yet lignified, giving the cellulose reaction. At this stage the fibers have reached their maximal development, for at the

---

*) protopectin in its proper sense,

**) Only in swollen walls the primary layer may be clearly distinguished from the middle lamella. In the descriptions of the anatomy and the microchemical reactions only the middle lamella and the thickening layers are mentioned in most cases. Therefore it is possible that on the one hand a part of the primary layer has been incorporated into the middle lamella and is on the other hand partly taken together with the thickening layers.

third and fourth stage they no longer increased in thickness and the lignin reaction remained the same.

Examining the higher parts of the stalks at these older stages the bast fiber cells are seen again in the various degrees of development and lignification already discussed above.

One or more rows of peculiar cells surround the bast bundles. At a transverse section these cells are much larger and more thin-walled than the bast fiber cells. They have, however, lignified walls which afterwards may be thickened and, some times, may lignify. It is remarkable that these lignified cells at the periphery of the bast bundles immediately adjoin the non-lignified parenchyma cells from which they are, in all other respects, indistinguishable. In some cases we found such cells at the periphery with lignified middle lamellae. The lignification occurred in the parts of the middle lamellae towards the bast bundle while the opposite parts were not lignified. We also found this phenomenon in longitudinal sections. But in general entirely lignified cells are adjoining non-lignified cells. The lignification process seems to proceed very rapidly through the middle lamellae all around the entire cell. The figure (fig. 9) shows a part of the bast bundle with the adjacent parenchyma cells.

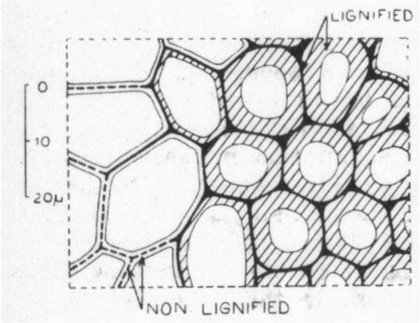


Fig. 9. Cross-section showing a part of a bast fiber bundle.

The transverse sections of the adult plants at the end of their life (series IV) draw our special attention. All internodes are full-grown so that mainly thickening processes have taken place. In the more basal parts the bast bundles look like those which are already discussed, but in the higher parts of the stalks complications occur in the whole xylem bundles. Not only a cap of heavily thickened elements is situated on the phloem side of the vascular bundle, but also a half ring of thick-walled prosenchymatous elements is situated on the xylem side pointing to the middle of the stalk. The vascular bundle is nearly entirely surrounded by a ring of thick-walled cells. At the outer- and innerside of the bundle a large cap of these elements and to the left and right side only one or two rows of these elements are present. On the outside of the ring of fibers a ring of two or three rows of very large, thin-walled, strongly lignified parenchyma cells are situated. Only their lignification sharply distinguishes them from the surrounding parenchyma cells. In some cases these transition cells are seen with partly lignified walls. In the internodes of the stalk just under the inflorescence the bast bundles have already thickened and lignified walls in contradistinction to those from the younger stages. The prosenchymatous elements form a nearly closed ring round the vascular bundles. The middle lamellae are strongly lignified while the thickening layers give a faint lignin reaction. At the young stages we have to deal with still growing parts. In the adult tissues the composition, and with that the properties of the walls, are only changed by chemical processes. Consequently, an interesting analogy exists in connection with the chemical composition. During the chemical analysis it appeared that the apical parts of plants of various ages, but not yet adult, had the same composition, while the apical part of the adult plants began to change its composition. The ageing process of the top of the stalk may be ascertained therefore microscopically as well as chemically.

We also investigated the course of development and lignification of the bast bundles in longitudinal sections and maceration preparations. Complete bast bundles were isolated from the stalks after maceration. At the base of the young hypocotyledon the bast bundles are rather small and consist of relatively short cells with thick, lignified walls provided with many pits. Upwards in the hypocotyledon the lignification becomes more intensive because, besides in the middle lamellae, lignin is also formed in the other thickening layers.

To the bundles new fibers are added, probably originating from daughter cells of an adjacent parenchyma cell. These daughter cells retain a small diameter. The ends of the fibers are mostly more or less sharp. At first these young cells are thin-walled and contain much protoplasm which remains in the lumen for a prolonged period. Thereupon the lignin reaction is soon seen in the middle lamellae, while at older stages the walls increase in thickness. Higher in the hypocotyledon the bast bundles become broader by the large number of fibers. The fibers are thick-walled with strong lignification in the middle lamellae and weak lignification in the thickening layers. This lignification decreases towards the lumen. In regard to the structure and number of fibers in the bast bundles the hypocotyledon possesses a more or less exceptional position, in as much as the fibers at the base of this internode are shorter and more pitted than at the top. Higher in the stalk the fibers are longer and contain only a few pits. It the other internodes the fibers are shortest at the top. In the younger internodes the walls of the fibers are not lignified and less thick. At the top the thin-walled elements mentioned above are present, differentiated from the further parenchyma. In the bast bundles isolated by maceration it may be clearly seen that the basal part is lignified and the apical part non-lignified. This basal part is stained dark red by phloroglucinol, and from base to top the colour fades more and more until it is wholly absent. With chlorzinc-iodide the base of the bundle is stained yellow and the top blue with all transitions from yellowish green to greenish blue between them.

In some longitudinal sections of the younger internodes, on the boundary of the bast bundles and the parenchyma, fibers are seen which show a lignified middle lamella towards the base of the stalks and a non-lignified one towards the top. The thickness of the wall was practically the same all over the fiber length, so that the lignified part of the wall was no more swollen than the non-lignified part. *Swelling (i.e. thickening) and lignification need not always to coincide.*

The non-lignified walls contain much pectin mainly localized in the middle lamellae. These layers swell considerably when placed in swelling-media and are stained intensively by ruthenium red. The lignified middle lamellae are still stained by this reagent, although in a lesser degree, and swell less strongly. The pectin is partly converted into — and consequently replaced by — the lignin. We must be very careful with the application of ruthenium red, for lignin also adsorbs this dye causing a red colouration. But generally a distinct difference is seen between the reaction on pectin and on lignin. When the former decreases the latter increases.

Summarizing we may say that in the bast bundles, also in the older stages, the strongest lignification is observed in the middle lamellae of the fibers. The thickening layers are always lignified less intensively and the layers close to the cell lumen hardly show any lignification. The cell walls do not always reach their definite thickness before the beginning of the lignification in the middle lamellae, but they often continue to thicken after the lignification of these lamellae and some times after the lignification also of the older thickening layers.

b. The wood vessels.

In transverse sections of the younger part of the stalks at various stages we meet the vascular bundles as groups of fan-shaped, diverging rows of elements situated in a non-lignified parenchyma, indistinguishable from the surrounding medullary parenchyma. We are dealing with protoxylem, a primary tissue originating from the vascular meristem. The number of vessels in a row amounts from 2 to 6, rapidly increasing in size in the radial direction towards the periphery of the stalk. In transverse sections various kinds of vessels are seen in such a row. The oldest vessels, being small and thick-walled, are situated most centrally in the stalk. Their wall thickenings appear to be

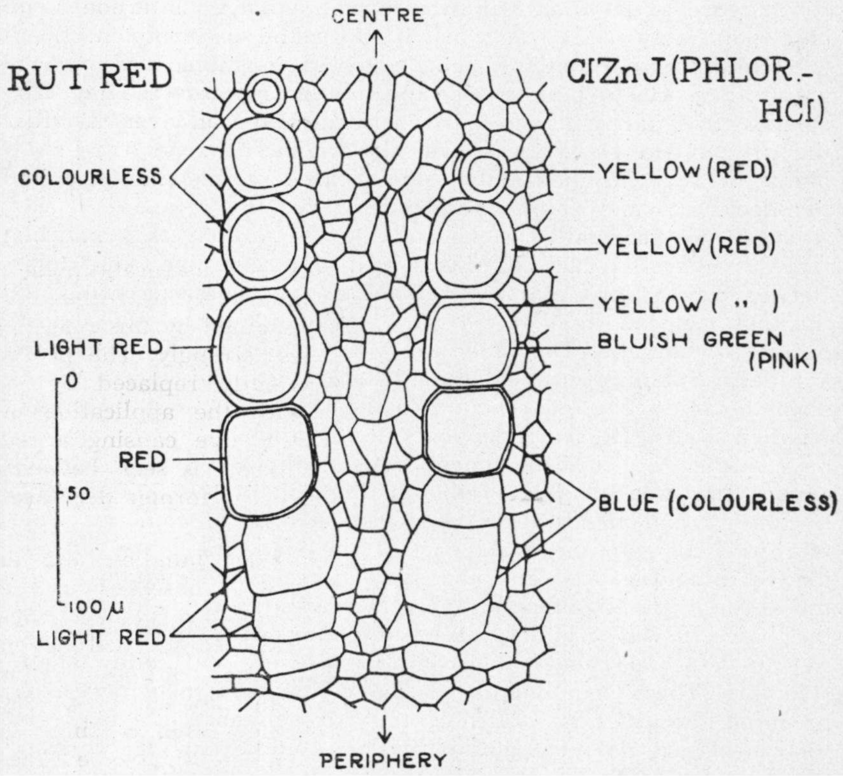


Fig. 10. Cross-section of a part of a young vascular bundle, stained with ruthenium red, chlorzinc-iodide or phloroglucinol-HCl.

strongly lignified (they are stained intensive yellow by chlorzinc-iodide and red by phloroglucinol-HCl, diffusely through out the whole wall). These vessels which appear to be ring vessels are followed by some vessels with a considerably greater diameter. They are provided with spiral thickening frames as is shown in the longitudinal sections. These frames are lignified less strongly. The next, still wider vessels have spiral and reticulate thickening frames which give a faint lignin reaction (faint red with phloroglucinol-HCl and greenish-blue with chlorzinc-iodide). The wide vessels situated nearest to the periphery do not yet show thickened wall frames and do not contain lignin. Examining the sections further from the top, the pictures appear to be the same, except for the presence of more vessels in a row. Figure 10 gives a clear picture of the reactions of the wall of the different vessels at various stages of development.

In the lower parts of the stalk, which shows already secondary growth, the xylem bundles become more complicated. They are composed of partly lignified parenchyma and, concomitant with the protoxylem elements, of vessels originating from the cambium. In such a bundle the oldest thick-walled and strongly lignified ring- and spiral vessels (the original "primanes") are situated most centrally in the stalk. Close to the cambium the largest vessels are to be found with thin, non-lignified walls. Between these two extremes elements are seen increasing in thickness and in lignification from the cambium to the oldest part of the bundle. The older the vessels the stronger they are lignified! Staining with chlorzinc-iodide all transitions are seen from the blue cellulose reaction to the yellow colour of strongly lignified walls. It clearly appears that various younger vessels have rather heavily thickened walls without showing lignification. This is a good proof that in the vessels the processes of thickening and lignification need not always to coincide. The more we come to the basal part of the stalk the more intense the cambium activity and the larger the xylem bundles. The intensity of the lignification also increases in the direction of the base. If we carefully macerate young parts of the stalks, we are able to isolate the complete vessels and study their structure. The xylem bundles of the young parts may be easily prepared, because the walls of the parenchyma cells between the vessels are not yet lignified and the middle lamellae contain much pectin being dissolved in the maceration by dilute hydrochloric acid. In this way we obtain series of vessels, situated side by side, showing a continuous transition from old, narrow and strongly lignified vessels to young, wide, non-lignified ones. The youngest vessels are filled with protoplasm, which exudes from the vessels when their joints cave in by a slight pressure on the elements (e.g. on the coverglass of the preparations). In some maceration preparations vessels with thickening frames were visible with a row of joints being not lignified in the direction of the top and a row of lignified joints in the direction of the base. The transition happened at two adjacent joints, but in a single case this transition was noticed within a joint, so that the lower part of this element was lignified and the upper part non-lignified. Occasionally one side of a thickening frame or ring was stained blue by chlorzinc-iodide and the other side yellow with greenish zones between them. This points to a local lignification within a single thickening ring.

In the apical part of series IV (the adult plant) the xylem bundles look a little different. As was noticed formerly, the thickened prosenchymatous elements are situated like a cap on the peripheral and on the central side of the vascular bundle. In the xylem part of the bundle ageing processes are going on. The wood parenchyma close to the cambium, unlignified at younger stages, starts to lignify at this stage. It is remarkable that the parenchyma situated a little further from the cambium has a small zone of cells not yet lignified.

We could explain this divergent behaviour by accepting that here no lignification should have taken place as long as the upper part of the stalk is still growing and the cambium is active. But no sooner the growth has stopped (the plant being full-grown) or the processes which affect lignification get free play and lignification may start here very energetically in the youngest part close to the cambium. The process is rapidly expanding perhaps because all kinds of factors have fallen away which should have hindered lignification in those cases where the cambium has still its full activity. In adult plants, showing no growth, the whole structure which controls the regular processes may be disturbed and a process such as lignification cannot longer proceed in the usual way.

The older the stalks are growing the more secondary wood is formed especially near the base. In this internode an entirely closed xylem ring will be produced. The large amount of newly formed vessels are chiefly pitted vessels as the elongation period is passed. The remnants of the protoxylem elements are pressed more and more into the secondary tissue so that here torn ring- and spiral vessels could be seen. Especially in the hypocotyledon of the stalk the process of lignification could be very distinctly examined at all stages.

If we study preparations treated with ruthenium red, it appears that the most strongly lignified vessels are not coloured, the weakly thickened and partly lignified vessels are pink and the hardly thickened, non-lignified vessels are red, while the thin-walled vessels close to the cambium are slightly stained. This fact leads to the supposition that cellulose should be present from the first (reaction with chlorzinc-iodide) and later on the cellulose becomes impregnated with pectic substances. A distinct relation may be seen between the ruthenium red reaction on the one side and the yellow chlorzinc-iodide reaction concomitant with the red phloroglucinol-HCl reaction on the other side. A strong pectin reaction coincides with a faint lignin reaction. This shows that layers rich in pectin do not contain lignin and that lignified walls contain little or no pectin.

c. The wood parenchyma.

In the young parts of the stalk no proper wood parenchyma is present. It is developed in the vascular bundles more towards the base. At first this wood parenchyma is still thin-walled, but afterwards it becomes thicker and begins to lignify.

The young parenchyma cells close to the cambium have no thickened and lignified walls, the cells away from the cambium have thicker walls which begin to lignify and the cells surrounding the oldest vessels have the most strongly thickened and lignified walls. We were able to follow the process very well by the use of chlorzinc-iodide. The entire cell wall in the youngest part of the parenchyma is stained blue; later on the middle lamellae are stained yellow and the further layers bluish. This blue turns yellow when the cells have become still older. With phloroglucinol-HCl these transitions are demonstrated in the various intensities of the red colour. This points to the fact that the lignification of these layers is proceeding slowly. Mostly the greater part of lignin is localized in the middle lamellae and only for a very small part in the thickening layers. In these layers the reaction with chlorzinc-iodide may turn from green to greenish-yellow, but never reaches the intensive yellow colour as can be observed in the middle lamellae of the cell walls. In many cases the inner thickening layer close to the cell lumen remains non-lignified (and is, consequently, stained blue by the reagent).

These modifications of the wood parenchyma are visible clearly in the hypocotyledons at the successive stages.

Apart from the lignified parenchyma cells in the xylem of the hypocotyledon at the older stages (series III and especially series IV) groups of very strongly thickened elements occur. They are called libriform or wood fibers and are not present in the younger hypocotyledons. They are produced later by the cambium together with secondary vessels and wood parenchyma cells. The lignin of these fibers is chiefly localized in the middle lamellae. The heavy thickening layers contain a very small amount of lignin. It seems that these layers are formed nearly exclusively by the deposition of cellulose with practically no lignin. Even the most heavily thickened fiber walls give no yellow but green colours with chlorzinc-iodide. Especially in the hypocotyledons of series IV the libriform fibers are developed very well. At the apices of these adult stalks lignified wood parenchyma is already present in the xylem bundle.

Examining the pectin reaction it appears that the non-lignified parenchyma walls are stained red especially in the middle lamellae and the weakly lignified walls are stained pink, while the colouration does not appear in the most strongly lignified walls. The pectinous middle lamellae easily swells in definite reagents and these swollen layers react intensively with ruthenium red. The course of lignification in the walls of parenchyma cells is the same as in the walls of bast fiber cells. The lignification begins in the middle lamellae where the greater part of the lignin is accumulated. Afterwards the process proceeds in the thickening layers of the walls with an decrease of its intensity from the middle lamellae towards the lumina of the cells. These layers are never lignified as strongly as the middle lamella.

Apart from the elements mentioned above lignification also occurs in the medullary parenchyma and medullary rays, in parts of the secondary bast parenchyma and even in parts of the phloem. These lignified elements can be seen in sections of stalks at the full-grown stage. Already in the top of these stalks the medullary parenchyma is partly lignified. In lower sections this is also the case with the secondary tissue situated on the outer side of the interfascicular cambium. In both cases we find large, and what is of great importance, thin-walled cells. From this it appears once more that lignification and thickening need not to coincide, so that the view that lignin formation should be a process of irreversible swelling is not always true. Concerning phenomena of lignification in the phloem we may refer to papers of BOODLE (12) and ALEXANDROV (2).

§ 2. *Microchemical examination.*

a. *Staining with oxamin blue.*

During lignification of the cell walls lignin is accumulated as an amorphous substance in the intermicellar spaces present between the cellulose crystallites.

The more lignin enters the spaces the less place is available for other substances. If we place transverse sections of the stalk in a solution of a metachromatic dye, various cell walls stain in a different manner. The colour of the cell walls depends upon the size of the particles which are taken up (CZAJA (23)). We used for our study oxamin blue which is such a poly-disperse dye.

In a transverse section near the top of a young stalk all elements were stained blue except for the lignified vessels. The young, thin-walled vessels became blue; the older vessels with thicker walls were stained red and the oldest, strongly lignified vessels were colourless to yellow. The non-lignified vessels took up in their walls the larger dye particles, staining blue. As a result of a partial lignification the entrance of blue particles was blocked and only the smaller particles, staining red, could penetrate the walls. In intensely lignified walls so much lignin was present that only the smallest particles, which cause a yellow colour (or no colouration at all), could be taken up.

In the heavy-walled lignified bast fiber cells at older stages we could observe that the middle lamellae adsorbed practically no dye (very strongly lignified!) and that the thickening layers were stained yellow. The colour was replaced by a reddish violet colour in less strongly lignified walls (e.g. from bast fibers at the edge of the bast bundles). The young, non-thickened and non-lignified cell walls of the wood parenchyma were coloured blue; the older, thickening and continually lignifying walls were purple, while the old, thick-walled, lignified parenchyma showed uncoloured middle lamellae and reddish, yellow or sometimes colourless thickening layers depending upon the degree of lignification.

With this staining method a very clear picture can be obtained of the proceeding lignification (ZIEGENSPECK (132), see also GRIFFIOEN (45)).

b. *Treatment with 72 per cent sulfuric acid.*

This treatment with sulfuric acid has been applied many times to examine the localization of lignin in the cell walls. Cellulose is hydrolysed and dissolved and lignin is left. With transverse sections we obtained chiefly the same pictures as already described in the literature. We want to indicate, however, some interesting points. After treatment of the strongly lignified, thick-walled bast fiber cells with concentrated H_2SO_4, the secondary thickening layers rapidly disappear without leaving a distinct trace. It has been an open question for a long time whether these layers do not contain any lignin or that this lignin has a special character, for these layers show mostly a very convincing lignin reaction. The middle lamellae of the cells do not dissolve in the sulfuric acid but are coloured yellowish brown as a result of some humification caused by the acid. The middle lamellae therefore contain very much lignin. The same result was obtained from the thick-walled and strongly lignified parenchyma cells. Here the middle lamella is left too, while the thickening layers are dissolved. If we examine the course of this reaction in a row of vessels on transverse sections of young parts of the stalk, the oldest and most strongly lignified walls of the vessels appear to remain unimpaired (save for a brownish humification). A little younger vessels are dissolved neither, but the weakly lignified vessels with rather heavy thickening frames on the walls show a remarkable swelling with sulfuric acid. The inner layers surrounding the lumen swell still stronger, begin to twist and thereupon disappear entirely, while the other layers dissolve and disappear gradually. In the young walls of the vessels little lignin but much cellulose and pectin are present. With sulfuric acid the layers swell up and the carbohydrates are hydrolysed. The small amount of lignin is not sufficient, however, to keep these layers together. In older vessels the walls are strongly lignified and hardly contain any pectin together with cellulose. The walls are not dissolved and by the larger amount of lignin present the structure seems to be unimpaired.

c. *Behaviour of the lignified elements against alkaline liquids.*

Lignin isolated from parts of the stalks at various ages did not always behave in a similar way towards alkaline solutions (Na-hydroxide, Na-carbonate and ammonia). They dissolved with more difficulty in these solutions as when they were obtained from older parts of the plants. Lignin isolated from the apical parts dissolved very easily, while the substance isolated from the basal part was slightly soluble. The decreasing solubility indicates an increase of the degree of polymerisation of the lignin. Young, newly formed lignin has a small molecule and dissolves very well, while old lignin is polymerized further and does not dissolve therefore easily. In this connection we want to call attention to the so-called saccharetine, a young lignin isolated from the sugar cane and easily soluble in alkalies (73). It is generally seen that very much lignin can be extracted from straw with Na-hydroxide at room temperature, while this procedure is realized for wood with more difficulties. In the latter case the treatment with Na-hydroxide must be very energetic. The studies of BECKMANN, LIESCHE and LEHMANN (10) and of METHA (78) are very interesting in this regard. So we meet in woods more polymerized lignins than in straw and perhaps in other herbaceous plants.

We wanted to investigate whether these characteristics also belong to the genuine lignin and therefore we treated transverse sections of the young stalks (with vessels as the only lignified elements) with the above-mentioned solutions. The sections were heated first with 8 per cent Na-hydroxide during about 5 minutes, washed and treated with phloroglucinol-HCl solution. The strongly swollen walls of the vessels did not show any trace of red colour. Treatment with 72 per cent H_2SO_4 brought the whole wall to solution without leaving a brown residu. *This clearly proved that the lignin had disappeared*!

In the youngest part of the stalks at older stages (e.g. in series IV) the lignin dissolved in the same way (after longer treatment with more concentrated alkali). From the older parts of the stalks the lignin could not be removed. Consequently genuine lignin reacts in the same way as isolated lignin with this difference, however, that lignins isolated from various stages dissolve more easily than the genuine lignins of the same stages.

Sections treated with dilute ammonia still gave a very distinct reaction on lignin which only disappeared after longer boiling with concentrated ammonia. (Less swelling occurs as after treatment with alkali.) Lignin from older stages could not be removed by ammonia.

After treatment with a 10 per cent solution of Na-carbonate the remaining mass still gave a distinct lignin reaction, although it was much weaker in consequence of the partial removal of the lignin.

We believe that the alkalinity of soda is too low to entirely remove the genuine lignin. Except for the gradual differences in solubility genuine lignins react in general in the same way as isolated lignins from the same parts of the plants.

d. *pH-determination as an indication for lignification.*

Miss MARTIN (77) has determined the hydrogen-ion concentration of various tissues of ***Helianthus annuus*** with the aid of the indicator method according to SMALL (121).

She found that lignified tissues were more acid than non-lignified. She estimated for xylem elements a pH = 4.0—4.4 but this pH may decrease even to 3.4 (lignified bast fibers!). The other elements are less acid, e.g. the cortex showed a pH = 5.6—6.0, while the highest value was 6.4 occurring in the sieve plates. We repeated these investigations with a large number of transverse sections of *Helianthus* at various ages, using six indicator solutions with a pH-range from 6.8—3.0, indicated by SMALL. We obtained chiefly the same results with our material. In the young parts of the top the strongly lignified walls of the oldest vessels showed the most acid reaction and the walls where lignification had proceeded less far were also less acid. The youngest vessels containing no lignin had the same pH as the cambium cells. We obtained similar results with the bast fibers as has been described already by Miss MARTIN. The proceeding lignification can be tested, therefore, very well by means of the increasing acidity of the elements.

§ 3. *Discussion.*

Summarizing the results of the microscopic examination we may say that lignification begins in the middle lamellae and reaches a great intensity at that place. Thereupon the process proceeds in the thickening layers without, however, reaching the intensity found in the middle lamellae. It appeared from various preparations that the walls still increase in thickness after the lignification of the middle lamellae (at this time the thickening layers do not contain lignin). Substances giving no lignin reaction are deposited from the protoplasm against or in the walls in the process of thickening. Besides the wall substances already present this newly deposited material will be partly converted into lignin.

Before the beginning of lignification the walls are composed of a number of substances present in a definite ratio. One or more of these wall substances — very probable pectic substances as appeared from the chemical and microscopic examination — are transformed into lignin. Meanwhile new material is supplied to the walls by the protoplasm and we assume (see page 364) that the composition and nature of this new material is similar to that of the original wall components. It seems that lignin matter as such does not enter the cell wall, in other words, is absent in the protoplasm. For, in the first place a lignin reaction never occurred in the protoplasm, (BOODLE (12), however, claimed to have detected this reaction in the cell contents of the phloem) and, moreover, secondly the lignin reaction decreases from the middle lamella in the direction of the lumen and finally disappears. If the lignin were present in the protoplasm, them the strongest reaction should be visible in the layers adjoining the protoplasm in the case of apposition of lignin or should be diffuse through the whole wall in the case of intussusception of this substance.

This train of thought could be criticized as the colour reactions on lignin are very unreliable. For example, it is possible that lignin is present in the protoplasm in a non-colourable form as a so-called protolignin with a small molecule. It is known too that some isolated lignins do not react with phloroglucinol-HCl.

It seems to be very doubtful, however, that by chance none of the numerous reagents should stain lignin in the protoplasm. *)

We have tried to elucidate in the chemical part of this study that pectins are very probable converted into lignin. The microscopic examination strengthened this supposition.

We want to mention here once more that the cell walls form a complicated system of very different substances which, therefore, may be hardly unravelled. The analysis is tried repeatedly but always has met with new difficulties. A good survey is given in a not very recent, but interesting, histological paper of METHA (78).

MANGIN (74) demonstrated by means of ruthenium red and maceration experiments that the middle lamella of young tissues is chiefly composed of pectin. Afterwards ALLEN (4) studied the pectinous character of the middle lamella more in detail and this character is since generally accepted by most investigators. The walls of the young cells need to be very plastic because these cells are still elongating. The presence of pectin in the middle lamella as a gelatinous, more or less plastic matter is therefore well conceivable. When the cells grow older such a condition is no longer necessary, even not desirable in connection with the

---

*) If the idea is true that living protoplasm is present everywhere in the cell walls, it is, of course, possible that lignin occurs in this protoplasm. But in that case the mechanism of all the alterations taking place in the cell walls seems to be still more complicated.

mechanical requirements of the plant. The gelatinous layer should be replaced by a solid lignified wall. Now it appears that wherever the lignin reaction is strong in the middle lamella, little or no pectin may be demonstrated and vice versa.

Especially the investigations of RITTER (97) have proved that the middle lamellae of the cell walls in the woody tissues are strongly lignified and hardly contain any pectin. HABERLANDT (46) alluded to such a lignification in his discovery that the middle lamellae of lignified elements are insoluble in concentrated H_2SO_4. HARLOW (47) investigated a large number of woods and young twigs on their wall substances with the same results. The middle lamella, at first rich in pectin, begins to lignify with decrease of the pectin content. JEFFREY (59) stated concerning the lignified middle lamella that the pectin of this layer "has undergone that somewhat complex and obscure modification chemically known as lignification". BAILEY (9), in his comprehensive researches, offered no opinion whether the polyuronides (pectins) are replaced by or transformed into lignin.

If we call, however, attention to the chemical possibilities of the pectin conversion and compare the regular relations existing between pectin and lignin at various growth stages, it cannot be expected that pectin disappears from the middle lamella (whether after decomposition or not), passes the other wall layers in any way, finally enters the protoplasm, is decomposed further or is accumulated elsewhere, while at the same time and with the same frequency other products from the protoplasm penetrate into the wall towards the middle lamella and form lignin in this lamella. *It is rational and in agreement with the observed facts that pectin is used for the lignin formation at the spot where it is deposited and that the factors required for this process are present there too.* In most cases some pectin remains after complete lignification (BAILEY). This untransformed pectin does not disappear but remains in the wall.

We may assume that lignification begins always in the middle lamellae affected by the vital processes of the cells. Afterwards the lignification is spread over the whole cell wall. In this respect the new view of TSCHIRCH (125) is mentioned. We may cite from his paper the following passage: "Die Tatsache, dass es die Intercellularsubstanz ist, die diese andere chemische Arbeit leistet (viz. formation of excretes, etc.) lenkt unsern Blick auf diese auch als Mittellamelle oder primäre Membran bezeichnete, die Zellen miteinander verkittende Schicht, von der wir längst wissen, dass sie sich chemisch anders verhält als die sogenannte sekundäre Membran". This "Intercellularsubstanz" should act "als dauernd am Leben bleibendes Biokolloid!" Moreover this substance should give a distinct lignin reaction in lignified elements! According to this view lignification, already seen as a necrobiotic phenomenon, should be carried out in a living layer outside the protoplasm. It is remarkable that **Tschirch** believes that this substance, which forms "ein Continuum durch die ganze Pflanze", is always living, the more as during strong lignification the cells should die according to the current ideas!

We do not believe the tenability of this idea of **Tschirch**, however, it very clearly demonstrates our scanty knowledge of the mechanism of the complicated lignification phenomena and other allied processes occurring in the cell walls.

In the thickening layers of the cell wall lignin may also be formed from pectin substances. In these layers the lignin reactions are mostly much fainter than in the middle lamella. **Ritter** (97) isolated this lignin with 72 per cent sulfuric acid and called it "cell wall lignin". On the contrary **Harlow** (48) believed that he dealt with decomposed products of the polysaccharides in the thickening layers caused by the action of strong acid! This idea, however, is untenable for it is in contradiction with the microchemical reactions performed with reagents without the use of strong acid. We can surely accept the presence of certain lignin components in the secondary layers and it is possible that these components are partly soluble in sulfuric acid and have disappeared after treatment with this acid.

The presence of pectic substances in the thickening layers is needed for lignin formation. **Mangin** has already demonstrated the widespread occurrence of these substances through the whole wall. Moreover, they are very distinctly demonstrated in the collenchyma cell walls (**Anderson** (5)) and in general the greater part of the cell walls are stained with ruthenium red, be it more or less faint according to the degree of lignification.

The tertiary lamella may also be lignified sometimes by the same processes. **O'Dwyer** (83) has demonstrated pectin in the tertiary lamella of the cell walls in beech wood and this pectin should be transformed into lignin in this layer. In other cases, however, this tertiary layer has a more gelatinous nature and consists of cellulose compounds (**Rendle** (95)).

The thickening frames in the vessels seem to be lignified in another way because these frames are equally stained and not in zones. We have to recognize that in this case the frames are wholly surrounded by the protoplasm. At the same time the products can be supplied from all directions and the factors which initiate or accompany lignification may collaborate. This is not the case with the usual wall thickenings where the supply is only possible from one side. Sometimes it is possible that the vessels are partly lignified either in some joints or within one cell.

The continuous proceeding of the lignification of the cell walls (e.g. the walls of the vessels) can be demonstrated very clearly by the decrease in dichroism present in cellulose walls after staining with chlorzinc-iodide, caused by the inpregnation of lignin. Strongly lignified walls show the dichroism no longer.

CHAPTER VII.

Some Biological Factors influencing Lignification.

In the literature many discrepancies are found concerning the internal and external factors affecting lignification. This seems to result partly from the fact that a proper theory of the formation of wood and lignin is not yet clearly defined.

The process of lignification is known to occur very early in the development of the plant. BURGERSTEIN (14) found in many plants that lignification often begins already on the second day after the appearance of roots or hypocotyledon. It is remarkable that lignification occurs so soon particularly in the walls of the vessels. That is why one has repeatedly asked for the significance of lignification (which, in most cases, takes place in special plant elements). It is, however, not possible to explain wholly the significance of the occurrence of lignin without entering in all kinds of speculative suppositions. Mostly no difference is made between the cause and the purpose of lignification and conclusions as to the significance of this phenomenon are very dangerous. For completeness' sake we want to discuss briefly some facts which have contributed to the more teleological theories of lignification. We believe, in contradistinction to other investigators, that, if we may speak about usefulness at all, we meet more than one function of the lignified walls.

It is remarkable that most investigators looked for a relation between the water economy and the lignification phenomena in the plant. Only those plants which need a special mode of the water transport show lignification of the walls. (Lower plants have, therefore, no lignified walls!) BURGERSTEIN already states that "die Holzstoff zur rascheren Leitung des Wassers durch die Wände der Zellgewebe wesentlich beiträgt". **Sachs** (101) assumes the extreme mobility of the water adsorbed, although the walls should be able to take up only a small amount of water; (afterwards it appeared, however, that the water is not transported through the walls but through the lumen of the vessels). **Porsch** (91) points to the large affinity of lignified walls for water; by this adhesion, added to the cohesion of the water itself, the formation of an unbroken water columm in the vessels is facilitated. **Jaccard** and **Frey** (58) note: "Die Elemente, welche in jungen Stengeln zuerst verholzen sind die wasserleitenden Tracheen. Der Wasserstrom im Leitungsgewebe beschleunigt also offenbar die Verholzung". Many objections may be raised against these theories. Various elements which have nothing to do with the water transport show lignification. Although the water plays a part in every living cell, some cell groups lignify rapidly, others slowly and others do not lignify at all. Moreover, it is very well possible that lignin or lignin-derivatives are also present in those plant groups where lignin could not be demonstrated up till now and where no special water supply through specific vessels takes place. In this case the relation between lignification and water transport should only be apparent.

Von Schellenberg (104) sees in lignification a kind of fixation of the cell walls whereby they lose the power of growth. That various adult cells are not lignified at all seems to contradict this idea. **Schilling** (105) claims to have observed abnormal growth in lignified tissues after injury but he did not keep in mind, however, that in consequence of the pathological state of the tissues delignification processes may take place (like these normally occurring in the stone cells of the pear, although in this case no cell division or growth takes place (1, 3)). **Molisch** (79) interpretes lignification as a means of the walls to resist biological attack since lignin, as an aromatic compound, in contradistinction to cellulose, is not decomposed by anaerobic micro-organisms and only by a special group of aerobic micro-organisms. This theory seems to be acceptable as its value is proved in nature as well as in laboratory. We may refer to a recent, instructive paper on the effect of lignin on fermentation of cellulose materials (**Olson** (84)). But here also objections may be raised as highly lignified elements do not resist to decomposition by certain micro-organisms. **Czapek** (24) found in sphagnol (in the cell walls of Musci) a typical analogon of

TABLE 13. Microchemical reactions on the cell walls at various degrees of lignification.

reagents	wood vessels			bast fiber cells			wood parenchyma	cambium	phloem	cortical parenchyma	medullary parenchyma
	very young	young	old	very young	young	old	young → old				
phloroglucinol-HCl	colourless	faint red	red	colourless	faint red	red	colourless → red				old pith is lignified locally
chlorzinc-iodide *	blue	green	yellow	blue	green	yellow	blue → yellow	blue	blue	blue	blue
ruthenium red **	red	light red	almost colourless	red	light red	colourless	red → light red	faint red	red	red	red
oxamin blue	blue	purple	yellow to colourless	blue	purple	yellow to colourless	blue → yellowish	blue	blue	blue	blue
72% sulfuric acid	dissolved	swollen and dissolved	not dissolved	dissolved	only middle lamellae not dissolved		only middle lamellae not dissolved				
Na-hydroxide	lignin dissolved	lignin not dissolved	lignin not dissolved								
ammonia	lignin dissolved	lignin not dissolved	lignin not dissolved								
Na-carbonate	lignin partly dissolved	lignin not dissolved	lignin not dissolved								
pH MARTIN		4.0—4.4			4.0—4.4		4.0—4.4	5.6—6.0	4.8—5.2	5.6—6.0	5.6—5.8
pH GRIFFIOEN	± 5.0	4.0—4.4		± 5.0	4.0—4.4		5.2 4.0—4.4				
rH tissues ***	almost wholly decoloured		little decoloured					wholly decoloured		partly decoloured	very little decoloured
rH transverse sections	strong reduction	faint reduction			faint reduction		faint reduction	very strong reduction	strong reduction	moderate reduction	very faint reduction

* in strongly lignified bast fibers and wood parenchyma cells mostly a greenish inner zone is present.

** the staining of the middle lamella is chiefly indicated.

*** rH determined with methylene blue solutions.

the preserving action of lignin. It is even probable that sphagnol is related to lignin (perhaps it might be considered as a protolignin).

FREY-WYSSLING (7) believes that lignification may be considered as a reaction against pressure. This might explain the occurrence of the first lignification phenomena in the thickening frames of young vessels, because these frames are exposed to a high lateral pressure caused by the turgor of the cells in the surrounding parenchyma. The so-called compression wood (Druckholz), subjected to high mechanical compression, lignifies more strongly than the so-called tension wood (Zugholz), subjected to mechanical tension. JACCARD and FREY (58) published an interesting paper on this matter.

FUCHS (43) also supposes that the occurrence of pressure during the process of lignification should have an important influence. He believes that the elements of higher plants which become vessels are subjected to pressure in the special conditions of land-life, which conditions cause lignification together with other phenomena.

In general it is not yet very plain which is the true significance of lignification for the mechanical properties of the plant. Non-lignified elements, for example, may be as rigid as lignified ones. Although we may ascribe a large compression strength to lignified tissues (according to JACCARD and FREY), many different opinions are put forward concerning various other properties, which are a consequence of the lignification.

SCHELLENBERG (104) found that lignified membranes show the same value of rigidity, elasticity and swelling power as non-lignified membranes while, on the contrary, SONNTAG (122) observed a decrease of these magnitudes during lignification.

The influence of nutrition factors on lignin formation was investigated by ANDRé (6), who discussed, moreover, the influence of water. In general the non-occurrence of lignification should be caused by an over-accumulation of nutriments. Furthermore, the formation of lignin as well as of secondary xylem should strongly decrease by a too low temperature, by diminition of the light intensity and by high air humidity (BERTHA ZINN (134)). These external factors affect of course finally the physiological state of the cells. Compare also the paper of GäUMANN (136).

Apart from these factors all kinds of specific internal factors must be present causing lignification only in specific cell walls. Unfortunately many internal conditions suitable for these processes are not yet known.

During our research we could detect one of these factors viz. the presence of a favourable reduction potential in the concerning cells.

It is seen that during lignification the carbon content of the walls increases considerably. Pectin, which has a low carbon content, is transformed into and replaced by lignin which is rich in carbon. This process should therefore require a strongly reducing environment and this condition should exist in the cambium and young xylem. The reduction potential in these tissues should be higher than in the surrounding tissues where such intensive reductions do not occur. In the strongly lignified tissues reductions are no longer required (moreover a large part of these cells is dead!). These conditions appeared to be very clearly realized in the material examined.

In living cells a large number of substances are present partly in a reduced and partly in an oxidized state, forming a labile equilibrium. A definite reduction potential (rH) is present in these living cells.

The rH-value is a concise expression of the oxidizing or reducing tendency of a chemical system, in terms of the pressure of gaseous hydrogen with which the system should be theoretically in equilibrium. The rH can be estimated electrometrically as well as colorimetrically. We applied the second method employing the so-called oxidation-reduction indicators, being dyes the solutions of which can be reduced to colourless leuko-compounds (13, 80). These compounds may be re-oxidized to the original dyes. We met here a reversible oxidation-reduction system. The depth of the colour of a solution of such an indicator depends upon the proportion of the oxidized and the reduced compound. A series of dyes exists which have each a different rH-range. Above this range they are totally coloured (oxidized) and below this range they are wholly decoloured (reduced).

For the determination of the rH of a tissue we have to employ a dye with about the same rH as that of the tissue. Moreover the dye solutions must be so diluted that the oxidation-reduction system of the material is not effected by that of the dye. Then it is necessary to work at a definite pH because the potential of the system is highly dependent upon the pH.

The investigations took place in two ways viz.

a) placing sections in dilute indicator solutions (microscopic method) and b) reducing these solutions by tissue-scrapings (macroscopic method). Both methods have many difficulties and there is no question of very accurate, quantitative results as, of course, the scraped, fresh tissues cannot be quantitatively weighed. Very interesting results are obtained, however, by these qualitative determinations. Employing the macroscopic method (with scraped masses of tissue) we worked as follows:

The hypocotyledons of young, well-developed stalks were taken because the active cambium has produced here much secondary wood. From the well-cleaned hypocotyledon the cortex was torn off along the thin-walled cambium. Now the cambial tissue situating against the xylem-ring and the same tissue joining the inner side of the torn-off cortex were carefully scraped with a clean piece of glass. These tissue-scrapings were placed in a tray with distilled water in order to remove the adhering liquids flown out of the damaged cells. Thereupon the remaining cortex as well as the wood cylinder were washed to remove remainders of the cambium and then the parenchyma was scraped from the cortex and from the xylem elements of the wood. Finally living medullary parenchyma from the inner part of the hypocotyledon was taken. All these tissues were separated from adhering impurities by washing with water and then divided for the experiments in as equal portions as possible. These experiments were carried out in so-called THUNBERG-tubes of thick glass, provided with a tap to be shut after evacuation. In each tube a portion of the tissue was placed, and 8 cc indicator solution and 2 cc buffer solution were added.

The indicator solutions were prepared in concentrations of 0.001—0.005 per cent by dissolving the dyes in neutral water (prepared by the mixture of tap water with distilled water up to a pH = 7). Solutions of 0.001—0.002 per cent gave the best results.

Phosphate buffers according to SÖRENSEN were used as buffer solutions. It appeared that pH = 7 was most suitable, so that this pH was always taken.

After being filled with solution and tissue the tubes were evacuated with a good air-pump. (This is necessary while otherwise the atmospheric oxygen re-oxidized the reduced dye.) After evacuation the taps were shut and the tubes placed in an incubator at 30° C. The reductions were realized rather slowly, for only after five or six hours a reaction was seen in dilute solutions. If we left the tubes in the incubator overnight, the reduction was already completed in most tubes so that no difference could be detected any more. Therefore the tubes were kept at room temperature overnight showing the next day colour variations in the solutions with different tissues.

Concomitant with the use of evacuated THUNBERG-tubes other test tubes were taken being not evacuated but shut with a layer of solid paraffin, after being entirely filled with the solutions. This method also yielded satisfactory results.

We started with a large series of indicators in order to find out the approximate rH-range of these living plant tissues. Solutions of o-cresol-indophenol (approximate rH-range from 18.5 to 20.5), tolylene blue (rH = 16—18) and brilliant cresyl blue (rH = 15—17) were very rapidly decolourized. This indicates that the oxidation-reduction potential of the tissues must be above these values. Methylene blue (rH = 13.5—15.5) is already decolourized with more difficulty but in most cases still by all tissues. Besides this indicator we employed a series of potassium indigo-sulphonates viz. di-, tri-, and tetrasulphonate with rH-ranges of resp. 8—10, 9.5—11.5 and 11—13. The reductions of these dyes succeeded with increasing difficulty; the disulphonate was only reduced in some cases. The reduced solutions being shaken with air or mixed with a drop of hydrogen peroxide turned again to the original colour.

Usually the reduction has proceeded most rapidly in the tubes with cambium tissue adjoining the xylem, while the cambium tissue near the phloem has a slightly weaker reducing action. The reduction caused by the cortical parenchyma is less strong and that of the xylem elements and the medullary parenchyma is weak. Tissues being previously killed with hot water showed no reduction power at all. Dead tissues were used therefore to check the results while the colours were compared with standard dye solutions.

We detected a great difference in reduction power between the cambium and the wood, the former being strongly reducing and the latter very weakly. *Consequently, we meet here one of the important factors playing a part during lignification: a reducing environment.* Moreover other specific factors will play a part as e.g. the cortex and the phloem do not lignify at once, although they have a rather high reducing power. It is difficult to express the rH of these tissues in exact numbers because the rH is very dependent upon the vitality of the plants used and it has appeared that this vitality varied widely at successive days. Of course the external factors play a great part here. For instance, a considerable difference in reduction intensity was seen between tissues of plants from the beginning of September, October or November. The latter showed almost no reduction power while the former were strongly reducing. It was remarkable that sometimes potassium indigo-trisulphonate was better

TABLE 14.
Determination of the reduction power in tissues of the sunflower.

Exp. 1, performed in THUNBERG-tubes on 28 September at 30° C from 11 h.—17 h.; overnight at 20° C till 29 September 10 h.

indicator solution	tissues	state of the solution
methylene blue: 8 cc of 0.0012% + 2 cc buffer (pH = 7)	cambium (xylem side)	totally decolourized
	cambium (phloem side)	almost „
	cortex	almost „
	xylem	weakly „
	medullary	weakly „

Exp. 2, performed in test tubes on 1 October at 30° C from 12 h.—17 h.; overnight at 20° C till 2 October 9 h.

indicator solution	tissues	state of the solution	
		at 17 h.	at 9 h.
methylene blue: 8 cc of 0.0025% + 2 cc buffer (pH = 7)	cambium	no reduction	total reduction
	cortex	„ „	little „
	control	„ „	no „
methylene blue: 8 cc of 0.0012% + 2 cc buffer (pH = 7)	cambium	beginning „	total „
	cortex	no „	¾ „
	control	„ „	no „
potassium indigo-tetrasulphonate: 8 cc of 0.002% + 2 cc buffer (pH = 7)	cambium	no „	weak „
	cortex	„ „	no „
	control	„ „	„ „

Exp. 3, without accurate indicator concentration but otherwise the same conditions as under Exp. 1.

indicator solution	tissues	state of the solution
methylene blue + buffer	cambium (living)	total reduction
	cambium (dead)	no „
	medullary (living)	partial „
	medullary (dead)	no „

reduced by the cambium tissue than tetrasulphonate. It even occurred that the cortex of the one plant reduced more strongly than the cambium of the other. It was, however, nearly always certain that the cambium reduced better than the other tissues of the same plant. The rH-values of the living tissues varied from about 9 to 15, the cambium having always the strongest reduction power and the wood the weakest. A table is given, showing the results of some of the numerous series obtained.

We also applied the second method with good results. Transverse sections of the stalks were placed in indicator solutions until they were stained well, thereupon washed in distilled water, mounted on hollow slides in a moist atmosphere, kept in an incubator at 30° C and examined from time to time. It is a pity, however, that no good limits of the rH-values could be found at all as the sulphonates were not taken up by the cell walls nor by the contents. For this reason these dyes were not suitable. Only methylene blue and brilliant cresyl blue could render us a service. The former was taken up mainly by the walls and the latter in the cell lumina. Both dyes were entirely reduced by the tissues except by the older xylem elements and the greater part of the medullary parenchyma. In spite of this rapid reduction the results were very interesting. Under the microscope it could be observed that already after a very short time (e.g. 15 minutes) the cambium was entirely decolourized, closely followed by the phloem. Thereupon the decolouration proceeded from the cambium through the cortex towards the periphery of the stalk, through the medullary rays to the central medulla and through the young xylem part. The oldest part of the xylem and the central part of the medulla retained their blue colour just as the most strongly lignified bast fibers (which are only stained by methylene blue!). It is remarkable that the starch sheath, in contradistinction to the further cortex, showed a very weak reduction potential (this was very clearly checked with brilliant cresyl blue). Already after some hours every reducing part of the stalk was decolourized. After addition of a droplet of hydrogen peroxide to these preparations the blue colour reappeared.

Also from these experiments it appears that the cambium possesses the most intensive reduction power, in agreement with our theory. We must point to the fact, however, that both methods have their inherent errors. The results are, therefore, more of a qualitative than of a quantitative nature. The material used was very heterogeneous because the tissue-scrapings were not identical. Moreover the tissue pieces form solid surfaces in the liquid, and of many solid surfaces the catalytic action on oxidation-reduction systems is known (KROON (69)).

It has to be borne in mind that the indicators may disturb the normal conditions in the cells and, moreover, these conditions are changed during the time in which the equilibria are established. It seems necessary to perform more experimental work in colorimetric rH-determination in tissues of higher plants.

The most important result of this study on the oxidation-reduction potential is the support it has given to our theory that lignification should be a reduction process. On spots where lignification begins reduction phenomena are seen to be really present. It is self-evident that with this fact the whole problem has not been solved because a large number of other factors, not yet detected, will affect the living substrate in which the processes occur.

This work was carried out in the Laboratory for Technical Botany of the University College of Technology at Delft.

I wish to express my grateful thanks to Prof. Dr L. G. M. Baas Becking for his invaluable help and constant interest in my study and to Prof. Dr Ir G. van Iterson for his important advice and the opportunity he has kindly given me to carry out these investigations in his laboratory.

SUMMARY.

1. A survey is given of the chemical composition of the various layers building up the cell wall in young, as well as in adult, stages.

2. An enumeration is given of the different and often discrepant theories on the origin of lignin.

3. In sunflower stalks at various growth stages determinations were carried out of lignin, cellulose, pectin, furfural-yielding components and of some substances not belonging to the wall.

4. The properties of the lignins isolated from these plant materials were studied and compared with the genuine lignins by means of microchemical reactions. Young lignins (from young parts of the stalk) are less polymerized than old lignins (from old parts of the stalks).

5. During the process of ageing and lignification the cellulose content remains nearly constant. The contents of lignin and

pentosan increase while the pectin percentage is rapidly decreasing. These results are observed. in a single stalk, proceeding from top to base, as well as in the same internode at successive growth stages.

6. Microscopic sections of the stalks were examined by application of microchemical reactions.

7. A close relationship is found to exist between pectin and lignin. If the pectin reactions are distinct, the lignin reactions are absent, and vice versa.

8. Special methods are described to determine the oxidation-reduction potential of living tissues of higher plants (in our case of the sunflower).

9. Especially the cambium and the adjacent layers of young xylem elements have a very strong reduction power.

10. An attempt has been made tot draw up an acceptable theory on lignin formation. According to this idea pectin should be converted into lignin, this being a strong reduction process. The factors needed for the realization of this reduction are present.

11. A part of the pectin, not taking part in lignin formation, may be changed into hemicelluloses (pentosans).

LITERATURE.

1. ALEXANDROV, W. G. and ALEXANDROVA, O. G. 1929. Ist die Verholzung ein reversibler oder irreversibler Vorgang? Planta 7, 340-346.
2. —— 1929. Ueber die Struktur verschiedener Abschnitte ein und desselben Bündels und den Bau von Bündeln verschiedener Internodien des Sonnenblumenstengels. Planta 8, 465-486.
3. ALEXANDROV, W. G. and DJAPARIDZE, L. I. 1927. Ueber das Entholzen und Verholzen der Zellhaut. Planta 4, 467-475.
4. ALLEN, C. E. 1901. On the origin and nature of the middle lamella. Bot. Gaz. 32, 1-34.
5. ANDERSON, D. B. 1927. Ueber die Struktur der Kollenchymzellwand auf Grund mikrochemischer Untersuchungen. Sitzber. Akad. Wiss. Wien. Math.-Naturw. Kl. I, 136, 429-439.
6. ANDRé, H. 1920. Ueber die Ursachen des periodischen Dickenwachstums des Stammes. Ztschr. f. Bot. 12, 177-218.
7. BAILEY, A. J. 1936. Lignin in Douglas fir. Ind. Eng. Chem. Anal. Edit. 8, 52-54 and 389-391.
8. —— 1936. The micro-determination of lignin. Mikrochem. N. F. 13, 98-107.
9. BAILEY, I. W. and KERR, TH. 1934. The cambium and its derivative tissues X. The structure of the so-called middle lamella. Jn. Arnold Arbor. 15, 233-254 and 327.

10. **Beckmann**, E., **Liesche**, O. and **Lehmann**, F. 1923. Qualitative und quantitative Unterschiede der Lignine einiger Holz- und Stroharten. Biochem. Ztschr. 139, 491-508.

11. **Bertrand**, G. and **Brooks**, G. 1937. Analyse et composition des tissus végétaux lignifiés. Bull. Inst. du Pin 27, 3e Série, 49-62.

12. **Boodle**, L. A. 1902 and 1906. On lignification in the phloem of *Helianthus annuus*. Ann. Bot. 16, 180-183; 20, 319-321.

13. British Drug Houses Analytical Laboratories. The colorimetric determination of the oxidation-reduction balance.

14. **Burgerstein**, A. 1874. Untersuchungen über das Vorkommen und die Entstehung des Holzstoffes in den Geweben der Pflanzen. Sitzber. Akad. Wiss. Wien. Math.-Naturw. Kl. I, 70, 338-355.

15. **Burkom**, J. H. **van**. 1913. Het verband tusschen den bladstand en de verdeeling van de groeisnelheid over den stengel. Thesis. Utrecht.

16. **Buston**. H. W. 1935. Observations on the nature, distribution and development of certain cell-wall constituents of plants. Biochem. Jn. 29, 196-218.

17. **Candlin**, E. J. and **Schryver**, S. B. 1928. Investigations of the cell-wall substances of plants, with a special reference to the chemical changes taking place during lignification. Proc. Royal Soc. London 103 B, 365-376.

18. **Cleve von Euler**, A. 1921 and 1922. Ueber dem Lignin nahestehende Harze und Gerbsäuren der Fichtennadeln. Cellulosechem. 2, 128-135; 3, 1-7.

19. **Cohen**, W. E. and **Harris**, E. E. 1937. Pretreatment of wood with hot dilute acid. Ind. Eng. Chem. Anal. Edit. 9, 234-235.

20. **Correns**, C. 1894. Ueber die vegetabilische Zellmembran. Jahrb. wiss. Bot. 26, 587-673.

21. **Crocker**, E. C. 1921. An experimental study of the significance of „lignin" color reactions. Jn. Ind. Eng. Chem. 13, 625-627.

22. **Cross**, C. F. and **Bevan**, E. J. 1918. Cellulose. London. p. 178-179.

23. **Czaja**, A. **Th**. 1930. Untersuchungen über metachromatische Färbungen von Pflanzengeweben. Planta 11, 582-626.

24. **Czapek**, **Fr**. 1899. Zur Chemie der Zellmembranen bei den Laub- und Lebermoosen. Flora 86, 361-381.

25. **Duncker**, B. J. J. 1935. Bouw en secundaire diktegroei van kruidachtige planten met interfasciculair cambium. Thesis. Utrecht.

26. **Ehrlich**, F. 1930. Ueber die Chemie des Pektins und seine Beziehungen zur Bildung der Inkrusten der Cellulose. Cellulosechem. 11, 140-151 and 161-170.

27. —— 1932. Pektin in **Klein**'s Handb. d. Pflanzenanalyse III, 1. Spez. Anal. II, 80-125.

28. **Eichler**, O. 1934 and 1935. Fluoreszensmikroskopische Untersuchungen über Verholzung und Lignin. Cellulosechem. 15, 114-124; 16, 1-7.

29. **Ender**, W. 1934. Die Entwicklung der Ligninchemie. Papierfabr. 32, 301-306.

30. **Faber**, F. C. **von**. 1904. Zur Verholzungsfrage. Ber. d. D. Bot. Ges. 22, 177-182.

31 **Farr**, W. K. and **Eckerson**, S. H. 1934. Formation of cellulose membranes by microscopic particles of uniform size in linear arrangement. Contrib. Boyce Thompson Inst. 6, 189, 309 and 315.

32. Falck, R. 1927. Der Lignin- und der Cellulose-Abbau des Holzes, u.s. w. Ber. d. D. Chem. Ges. 60, I, 225-232.

33. Fellenberg, Th. von. 1918. Ueber die Konstitution der Pektinkörper. Biochem. Ztschr. 85, 45-118.

34. Franken, H. A. 1934. Voorkomen, bereiding en eigenschappen van de, in de natuur aangetroffen, uronzuren en van enkele verwante zuren. Thesis. Delft.

35. Fremy, E. 1876. Méthode générale d'analyse du tissu des végétaux. Etudes chimiques sur le squelette des végétaux. Ann. Sc. nat. Sér. 6 (bot.), 13, 353-382.

36. Friesen, G. 1935. Kritische Untersuchungen über den Nachweis von Ligninen in Zellwänden. Ber. d. D. Bot. Ges. 53, 186-196.

37. Freudenberg, K. 1933. Tannin, Cellulose und Lignin. Berlin.

38. Freudenberg, K. and Duerr, W. 1932. Konstitution und Morphologie des Lignins. Klein's Handb. d. Pflanzenanalyse III, 1. Spez. Anal. II, 125-156.

39. Frey-Wyssling, A. 1935. Die Stoffausscheidung der höheren Pflanzen. Berlin.

40. Friedrich, A. and Diwald, J. 1925. Zur Kenntnis des Lignins I. Sitzber. Akad. Wiss. Wien. Abt. II B, 134, 31-46.

41. Friedrich, A. and Salzberger, A. 1929. Zur Kenntnis des Lignins V. Sitzber. Akad. Wiss. Wien. Abt. II B, 138 (Suppl.), 989-1001.

42. Fuchs, W. 1926. Die Chemie des Lignins. Berlin.

43. —— 1926. Theorie der Ligninbildung. Biochem. Ztschr. 180, 30-35.

44. —— 1931. Untersuchungen über Lignin, Huminsäuren und Humine. Ztschr. angew. Chem. 44, 111-118.

45. Griffioen, K. 1934. A study on the dark coloured duramen of ebony. Rec. Trav. Bot. Néerl. 31, 780-809.

46. Haberlandt, G. F. J. 1904. Physiologische Pflanzenanatomie. Leipzig.

47. Harlow, W. M. 1927. The chemical nature of the middle lamella. Techn. Publ. N. Y. State Coll. Forestry Syracuse. 27, 5-11.

48. —— 1932. Contributions to the chemistry of the plant cell wall VI. Further studies on the location of lignin in the cell walls of wood. Amer. Jn. Bot. 19, 729-739.

49. Harris, E. E., Sherrard, E. G. and Mitchell, R. L. 1934. Some chemical reactions of maple and spruce lignin. Jn. Amer. Chem. Soc. 56, 889-893.

50. Hawley, L. F. 1932. The differentiation of hemicelluloses. Ind. Eng. Chem 24, 1190-1194.

51. Hawley, L. F. and Harris, E. E. 1932. Synthetic lignin. Ind. Eng. Chem. 24, 873-875.

52. Hempel, H. 1934. Die chemischen Aufbaustufen der Holzschichten. Cellulosechem. 15, 41-43.

53. Hess, K., Trogus, C. and Wergin, W. 1936. Untersuchungen über die Bildung der Pflanzlichen Zellwand. Planta 25, 419-437.

54. Hilpert, R. S. 1935. Ueber Lignin in Stroh und Laubhölzern. Cellulose chem. 16, 92-96.

55. Hilpert, R. S. and Littmann, E. 1934. Ueber die Verharzung der Zucker durch Säuren und ihre Beziehung zur Ligninbildung. Ber. d. D. Chem. Ges. 67 B, 1551-1556.

56. —— 1935. Ueber Lignin-Bestimmung bei tiefen Temperaturen und die vollständige Hydrolyse des Strohes. Ber. d. D. Chem. Ges. 68 B, 16-18.

57. Hilpert, R. S. and Hellwage, H. 1935. Buchenholz-Lignin. Ein Reaktionsprodukt der Kohlehydrate bei der Lignin-Bestimmung. Ber. d. D. Chem. Ges. 68 B, 380-383.
58. Jaccard, P. and Frey, A. 1928. Einfluss von mechanischen Beanspruchungen auf die Micellarstruktur, Verholzung und Lebensdauer der Zug- und Druckholzelemente beim Dickenwachstum der Bäume. Jahrb. wiss. Bot. 68, 844-866.
59. Jeffrey, E. C. 1917. The anatomy of woody plants. Chicago. p. 6.
60. Jonas, K. G. 1921. Zur Kenntnis der Lignin- und Huminsubstanzen. Ztschr. angew. Chem. 34, 289-291.
61. Kalb, L. 1932. Analyse des Lignins. Klein's Handb. d. Pflanzenanalyse III, 1. Spez. Anal. II, 156-204.
62. Klason, P. 1908. Hauptsammlungsbericht d. Vereins d. Zellstoff- und Papierchemiker, Berlin s. 52. (See Cellulosechem. 4, 81).
63. —— 1932. Zusammenfassung von Untersuchungen in der Chemie des Nadelholzes. Cellulosechem. 13, 113-119.
64. —— 1934. Untersuchungen über den Zusammenhang zwischen dem natürlichen System der Pflanzen und ihrem chemischen Bau. Svensk Papp. Tidn. 37, 302. Ref. Cellulosechem. 15, 42.
65. —— 1936. Ueber die Bildung von Lignin im Holz. Ber. d. D. Chem. Ges. 69 B, 676-678.
66. Klatt, W. 1935. Quantitative Lignin-Bestimmung kleinster Holzmengen mittels Fluorwasserstoff. Angew. Chem. 48, 112.
67. Koenig, J. and Becker, E. 1919. Die Bestandteile des Holzes und ihre wirtschaftliche Verwertung. Ztschr. angew. Chem. 32, 155-160.
68. Koenig, J. and Rump, E. 1914. Chemie und Struktur der Pflanzen-Zellmembran. Berlin.
69. Kroon, D. B. 1935. Oxydatie-katalyse aan grensvlakken. Thesis. A'dam.
70. Kuerschner, K. See W. Fuchs, Die Chemie des Lignins. 1926.
71. —— 1934. Techn. 31. 24. (not seen).
72. —— 1935 and 1936. Schwankende Grundlagen der gesammten Ligninchemie? Zellstoff-Faser 32, 132-134 and 33, 49-54.
73. Langguth-Steurwald, L. G. 1911. Over de incrusteerende kleurstof van het suikerriet. Meded. Proefst. Javasuikerindustrie 12, 365-379.
74. Mangin, L. 1889. Sur la présence des composés pectiques dans les végétaux. Compt. Rend. 109, 579-582.
75. —— 1893. Sur l'emploi du rouge de ruthénium en anatomie végétale. Compt. Rend. 116, 653-656.
76. Marcusson, J. 1925. Die Struktur der Huminsäuren und Kohlen. Ber. d. D. Chem. Ges. 58, 869.
77. Martin, S. H. 1927. The Hydrion Concentration of plant tissues III. The tissues of *Helianthus annuus*. Protoplasma 1, 497-536.
78. Metha, M. M. 1925. Biochemical and histological studies of lignification. Biochem. Jn. 19, 958-997.
79. Molisch, H. 1932. Ueber die Bedeutung des Lignins für die Pflanze. Ztschr. f. Bot. 25, 583-595.
80. Needham, J. and Needham, D. M. 1927. The oxidation-reduction potential of protoplasm. Protoplasma 1, 255.
81. Norman, A. G. and Jenkins, S. H. 1934. The determination of lignin I and II. Biochem. Jn. 28, 2147-2159 and 2160-2168.
82. Oden, S. 1926. Om Ligninets Uppkomst och Omvandling i Växtriket. Svensk Kem. Tidskr. 38, 122-130 (see Fuchs, l. c.).

83. O'Dwyer, M. H. 1925. A note on the occurrence of a pectic substance in beech wood. Biochem. Jn. 19, 694-696.

84. Olson, F. R., Peterson, W. H. and Sherrard, E. C. 1937. Effect of lignin on fermentation of cellulosic materials. Ind. Eng. Chem. 29, 1026-1029.

85. Ost, H. and Wilkening, L. 1910. Die Verzuckerung des Zellstoffs. Chem. Zt. 34, 461-462.

86. Paloheimo, L. 1929. Beiträge zur Ligninbestimmung mit Säurehydrolyse. Biochem. Ztschr. 214, 161-174.

87. Pauly, H. and co-operators. 1934. Scheidung von Lignin-Komponenten. Ber. d. D. Chem. Ges. 67 B, 1177-1199.

88. Payen, M. 1839. Mémoire sur la composition du tissu propre des plantes et du ligneux. Ann. Sc. nat. Sér. 2 (bot.), 11, 21-31.

89. Phillips, M. and Goss, M. J. 1934. Lignin IX. Lignin from barley straw. Jn. Amer. Chem. Soc. 56, 2707-2710.

90. —— 1935. Composition of the leaves and stalks of barley at successive stages of growth, with special reference to the formation of lignin. Jn. Agricult. Research 51, 301-319.

91. Porsch, O. 1926. Zur physiologischen Bedeutung der Verholzung. Ber. d. D. Bot. Ges. 44, 137-142.

92. Rassow, B. and Gabriel, H. 1931. Ueber das Fichtenholz-Lignin. Cellulosechem. 12, 227-235.

93. Rassow, B. and Zschenderlein, A. 1921. Ueber die Natur des Holzes des Hanfes. Ztschr. angew. Chem. 34, 204-206.

94. Reed, H. S. and Holland, R. H. 1919. The growth rate of an annual plant *Helianthus*. Proc. Nat. Acad. Sci. 5, 135-144.

95. Rendle, B. J. 1937. Gelatinous wood fibres. Trop. Woods 52, 11-19.

96. Richter, G. A. 1931. Purified wood fiber. A study of the physical and chemical properties. Ind. Eng. Chem. 23, 131-139.

97. Ritter, G. J. 1925. Distribution of lignin in wood. Ind. Eng. Chem. 17, 1194-1197.

98. Ritter, G. J. and Kurth, E. F. 1933. Holocellulose, total carbohydrate fraction of extractive-free maple wood. Ind. Eng. Chem. 25, 1250-1253.

99. Ritter, G. J., Seborg, R. M. and Mitchell, R. L. 1932. Factors affecting quantitative determination of lignin by 72 per cent sulfuric acid method. Ind. Eng. Chem. Anal. Edit. 4, 202-204.

100. Ruge, U. 1937. Ueber einige Alterungserscheinungen in der Intermizellarsubstanz junger, streckungsfähiger Membranen. Planta 27, 436-449.

101. Sachs, J. 1892. Ueber die Porosität des Holzes. Gesam. Abh. ü. Pflanzenphysiologie. 1, 510-553.

102. Sanio, C., 1863. Vergleichende Untersuchungen über die Elementarorgane des Holzkörpers. Bot. Zt. 21, p. 93 and 104.

103. Scarth, G. W., Gibbs, R. D. and Spier, J. D. 1929. Studies of the cell wall in wood. Proc. Royal Soc. Canada 23, 3 d Series, 269-288.

104. Schellenberg, H. 1896. Beiträge zur Kenntnis der verholzten Zellmembran. Jahrb. wiss. Bot. 29, 237-266.

105. Schilling, E. 1923. Ein Beitrag zur Physiologie der Verholzung und des Wundreizes. Jahrb. wiss. Bot. 62, 528-562.

106. Schindler, H. 1931. Kritische Beiträge zur Kenntnis der sogenannten Holzreaktionen. Ztschr. wiss. Mikrosk. 48, 289-319.

107. Schmidt, E. 1936. Ueber die Frage: „Was ist Lignin?" Papierfabr. 34, 161-163.

108. Schmidt-Nielsen, S. and Opperud, A. 1935. Carbohydrates as sources of error in the lignin determination. Ref. Chem. Abstr. 31, 2419, 1937.

109. Schmidt-Nielsen, S. and Astad, A. 1935. „Lignin" of seaweeds. Ref. Chem. Abstr. 31, 2419, 1937.

110. Schrauth, W. 1923. Ueber das Lignin. Ztschr. angew. Chem. 36, 149-152.

111. Schulze, F. 1857. Chem. Zentralbl. 28, 321.

112. Schwalbe, C. G. 1936. Zum Ligninproblem. Cellulosechem. 17, 113-123.

113. Schwalbe, C. G. and Becker, E. 1920. Die chemische Zusammensetzung des Erlenholzes. Ztschr. angew. Chem. 33, 14-16.

114. Schwalbe, C. G. and Neumann, K. E. 1931. Der Sauerstoff als wesentlicher Faktor bei der Holzbildung. Cellulosechem. 12, 287-290.

115. —— 1933. Der Luftsauerstoff, ein Faktor bei der Holzbildung. Cellulosechem. 14, 133-134.

116. Seifriz, W. 1934. The origin, composition and structure of cellulose in living plants. Protoplasma 21, 129-159.

117. Shaw, R. H. and Wright, P. A. 1920. A comparative study of the composition of the sunflowers and corn plants of different stages of growth. Jn. Agricult. Research 20, 787-793.

118. Sherrard, E. C. and Harris, E. E. 1932. Factors influencing properties of isolated wood lignin. Ind. Eng. Chem. 24, 103-106.

119. Sloep, A. C. 1928. Onderzoekingen over pectinestoffen en hare enzymatische ontleding. Thesis. Delft.

120. Sloep, A. C. 1937. Die wissenschaftlichen Grundlagen zur Standardisierung der Pektin-Analyse. C. R. Ve Congr. Int. Techn. et Chim. Ind. Agric. Schéveningue. 141-171.

121. Small, J. 1929. Hydrogen-ion concentration in plant cells and tissues. Protoplasma Monographien II, Berlin.

122. Sonntag, P. 1901. Verholzung und mechanische Eigenschaften der Zellwände. Ber. d. D. Bot. Ges. 19, 138-149.

123. Stamm, A. J., Semb, J. and Harris, E. E. 1932. Absorption spectra of lignin solutions. Jn. Phys. Chem. 36 I, 1574-1584.

124. Staudinger, H. and Dreher, E. 1936. Ueber hochpolymere Verbindungen. Ueber das Lignin. Ber. d. D. Chem. Ges. 69 B, 1729-1737.

125. Tschirch, A. 1934. Ueber Arbeitsteilung im Chemismus der höheren Pflanzen. Helv. Chim. Acta 17, 992-996.

126. Waksman, S. A. and Cordon, T. C. 1936. On the nature of lignin. Jn. Amer. Chem. Soc. 58, 969-971.

127. Waksman, S. A. and Stevens, K. R. 1929. Processes involved in the decomposition of wood with reference to the chemical composition of fossilized wood. Jn. Amer. Chem. Soc. 51, 1187-1196.

128. Wehmer, C. 1915. Zum Abbau der Holzsubstanz durch Pilze. Ber. d. D. Chem. Ges. 48 I, 130-134.

129. Wislicenus, H. 1933. Ueber Ur-Cellulose und Ur-Lignin in den holzbildenden Pflanzensäften als chemische Baustoffe der Holzsubstanz. Papierfabr. 31, Fest- und Auslandh. 65, 65-77.

130. Wislicenus, H. and Hempel, H. 1933. Zur Kenntnis des stofflichen Aufbaues der Holzsubstanz aus den holzbildenden Säften der Baumpflanze. Fruktose als Urbaustoff des „Lignins". Cellulosechem. 14, 149-168.

131. Ziegenspeck, H. 1925. Ueber Zwischenprodukte des Aufbaues von Kohlenhydrat-Zellwänden und deren mechanischen Eigenschaften. Bot. Arch. 9, 297-376.

132. —— 1931. Ueber die Zwischenzustände bei der Bildung verholzter Membranen. Ber. d. B. Bot. Ges. 49. 381-386.

133. Zherebov, L. P. 1936. Formation of lignin. Bumazhnaya Prom. 15, 27-37. Ref. Chem. Abstr. 31, 2418, 1937.

134. Zinn, B. 1930. Abhängigkeit der Lignin- und Xylembildung von äusseren Faktoren. Thesis. Basel.

135. 1936. Neuere Ergebnisse der reinen und angewandten Holzforschung. Zellstoff-Faser 33, 89-93, 105-110 and 166-168.

136. Gäumann, E. 1932. Der Einfluss der Keimungstemperatur auf die chemische Zusammensetzung der Getreidekeimlinge I. Ztschr. f. Bot. 25, 385-461.