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Studies on Compression Wood — Part VII:

Distribution of Lignin in Normal and Compression Wood of Tamarack*

[*Larix laricina (Du Roi) K. Koch*]

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Summary

The distribution of lignin has been studied in tracheids and ray cells of normal and compression wood of tamarack [*Larix laricina* (Du Roi) K. Koch]. The three layers in the secondary wall of normal wood tracheids are lignified to approximately the same extent, and previous evidence that the S_3 layer should contain a higher proportion of lignin than the other regions has not been confirmed. The lignin follows closely the orientation of the cellulose microfibrils in all three layers. Compared to the tracheids, the ray cells contain a denser network of lignin in their secondary wall.

Only a small proportion of the total lignin in compression wood tracheids is present in the compound middle lamella. The thick S_1 layer is only slightly lignified; the orientation of the lignin in this region is that of the transversely oriented, lamellated microfibrils. The outer portion of S_2 consists largely of lignin but also contains lamellae of cellulose microfibrils which probably have the same helical orientation as the microfibrils in the inner part of S_2. The latter region, which contains the helical cavities, consists of lamellae of cellulose microfibrils which are uniformly encrusted with lignin. The ray cells in compression wood appear to be lignified to the same extent as in normal wood. Transverse sections of the cells reveal a lateral orientation of the lignin. The orientation of the cellulose microfibrils in the S_2 layer of the first-formed springwood tracheids of compression wood is the same as in the cells which are formed later. It is suggested that for ease of reference, the outer, lignin-rich layer in compression wood tracheids be referred to as the $S_2(L)$ layer.

Zusammenfassung

Im Druckholz und im normalen Holz von Tamarack (*Larix laricina* (Du Roi) K. Koch) wurde die Verteilung des Lignins in Tracheiden und Markstrahlzellen untersucht. Die drei Schichten der Sekundärwand in den Tracheiden normalen Holzes werden in nahezu demselben Umfange lignifiziert. Frühere Feststellungen, daß die S_3-Schicht einen höheren Ligningehalt erreicht als andere Zellwandbereiche, konnten also nicht bestätigt werden. Das Lignin folgt sehr genau der Orientierung der Cellulose-Mikrofibrillen aller drei Schichten. Im Vergleich zu den Tracheiden erfahren die Sekundärwände der Markstrahlzellen eine stärkere Ligninauskleidung.

Nur ein geringer Prozentsatz des gesamten Lignins der Druckholztracheiden befindet sich in der Mittellamelle. Die dicke S_1-Schicht ist nur wenig lignifiziert. Die Orientierung des Lignins in diesem Bereich entspricht den transversal orientierten, lamellierten Mikrofibrillen. Der äußere Teil der S_2-Schicht enthält sehr viel Lignin, daneben aber auch Lamellen von Cellulose-Mikrofibrillen, die wahrscheinlich dieselbe spiralige Orientierung besitzen wie die Mikrofibrillen des inneren Teiles der S_2-Schicht. Der letzterwähnte Bereich, der spiralige Kavitäten enthält,

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weist Lamellen von Cellulose-Mikrofibrillen auf, in welche gleichmäßig Lignin eingelagert ist. Die Markstrahlzellen des Druckholzes erscheinen ebenso stark lignifiziert wie die Markstrahlzellen des Normalholzes. Querschnitte durch diese Zellen lassen die laterale Orientierung des Lignins erkennen. Die Orientierung der Cellulose-Mikrofibrillen in der S_2-Schicht der zuerst gebildeten Frühholztracheiden des Druckholzes ist dieselbe wie in jenen Zellen, die später ausgeformt werden. Es wird vorgeschlagen, daß zur eindeutigeren Kennzeichnung die äußere ligninreiche Schicht der Druckholztracheiden als $S_2(L)$-Schicht bezeichnet wird.

Introduction

The distribution of lignin in wood, and especially in wood from conifers, has attracted attention ever since the pioneering investigations of RITTER [1925, 1928], HARLOW [1928, 1931, 1939], and DADSWELL [1931]. Earlier contributions were recently reviewed by CÔTÉ, TIMELL, and ZABEL [1966]. Two years ago, BERLYN and MARK [1965], in an important contribution, drew attention to the fact that, contrary to previous belief, less than 40% of the total lignin could be located in the compound middle lamella (M + P), the majority actually being found in the secondary wall of a conifer (softwood) tracheid. Direct measurements by ultraviolet spectroscopy have lately led GORING [unpublished] to the same conclusion.

It has been known for almost 50 years that compression wood contains considerably more lignin than corresponding, normal wood. BAILEY and KERR [1937] and DADSWELL and ELLIS [1940] noticed that in transverse sections of tracheids from compression wood, the lignin appeared to be organized in a radial pattern as contrasted to the concentric arrangement in normal wood. WARDROP and DADSWELL [1950] removed the polysaccharides from compression wood of *Pinus pinaster* and *Araucaria cunninghammi* by treatment with 72% sulfuric acid. Examination by light microscopy of the remaining lignin revealed a radial distribution in radial and a spiral one in tangential sections. In the former species, which possessed coarse spiral striations in the cell wall, the lignin pattern was coarse, while in the latter, which had smaller helical cavities, the lignin pattern was finer. It was concluded that, because the cellulose microfibrils run parallel to the helical ribs, the lignin distribution will appear radial in transverse and spiral in longitudinal sections. This interpretation would seem to be correct, the arrangement of the lignin skeletons merely reflecting the organization of the original S_2 layer.

BAILEY and BERKELEY [1942] noted that compression wood tracheids from *Taxodium distichum* contained two layers; in the outer the fibrillar orientation was largely transverse, while the inner layer contained radio-helical bands oriented at an angle of 44° ... 48° to the fiber axis. Between the two layers there was a region which in cross-section was isotropic or only feebly birefringent in polarized light, a property which the investigators felt was due to the presence of a high proportion of non-cellulosic constituents, rather than to the occurrence of longitudinally arranged fibrils.

The two layers referred to by BAILEY and BERKLEY [1942] were later identified by WARDROP and DADSWELL [1950] as S_1 and S_2, respectively. The existence of an interposed, isotropic layer could not be confirmed for *Taxodium distichum* although some evidence was obtained for its occurrence in *Pinus pinaster*, *Pinus radiata*, and *Pseudotsuga menziesii*. WARDROP and DADSWELL [1950) concluded

that the presence of a non-cellulosic region between S_1 and S_2 could not be deemed characteristic of compression wood tracheids.

LANGE [1954] found that compression tracheids of *Picea abies* contain a narrow region between the two secondary walls, a region which has a relatively high concentration of lignin and which forms a system of adjacent, hollow tubes. The inner layer, on the other hand, was stated to be practically free of lignin. RUCH and HENGARTNER [1960] have criticised the microspectrographic technique used by LANGE. WERGIN [1965] has pointed out that the cells studied by LANGE were not typical of those usually found in compression wood since they lacked a rounded outline, intercellular spaces, and helical cavities in S_2. Inspection of LANGE's photomicrograph reveals that this is correct. The ultraviolet light transmission across the middle lamella and cell walls was, nevertheless, quite different from that given by normal wood tracheids. In view of the recent results of CÔTÉ, KUTSCHA, SIMSON, and TIMELL [1968], it is possible that the tracheids studied by LANGE had been formed at the beginning of the growing season, when compression wood cells often lack the above characteristics but differ from normal cells in their chemical composition and lignin distribution.

Examining compression wood tracheids of *Pinus radiata*, WARDROP and DAVIES [1964] noted between S_1 and S_2 a region of very low birefringence which also had a high ultraviolet absorption. It could be shown by electron microscopy that this zone did not contain any steeply oriented microfibrils which could have caused extinction. Instead it was concluded that the weakly birefringent zone, while containing some lamellae of cellulose, consisted largely of lignin, thus confirming the observations of BAILEY and BERKLEY [1942].

A different picture of the distribution of lignin in compression wood tracheids has been given by CASPERSON [1959, 1963, 1965], who is of the opinion that the larger part of the lignin is located in the middle lamella, in the primary wall and in the S_1 (*"transition lamella"*). On treatment with chlorous acid, the lignin in these regions, and especially that in S_1 was rapidly removed, while that in S_2 was least attacked. Removal of the lignin left microfibrils of cellulose in the S_1 layer, which, according to CASPERSON [1965] is more highly lignified than in normal wood.

WERGIN [1965] studied the distribution of lignin in compression wood of *Picea abies* by ultraviolet light microscopy. The primary wall was found to be unusually wide and showed only slight absorption in ultraviolet. A thin, strongly absorbing layer was identified as S_1. The remainder of the secondary wall exhibited fairly strong absorption. Extinction curves were obtained for both normal and compression wood. It was concluded that the primary wall in compression wood is unusually wide and is less highly lignified than in normal wood, that the S_1 layer is heavily lignified, and that the remainder of the secondary wall, contrary to the opinion of LANGE [1954], contains a considerable amount of lignin.

In their study on the distribution of lignin in normal and compression wood tracheids of *Picea rubens* and *Abies balsamea*, CÔTÉ, TIMELL, and ZABEL [1966] arrived at conclusions quite different from those of CASPERSON [1963] and WERGIN [1965]. In this investigation, polysaccharides were removed either chemically or enzymically, and the texture of the remaining lignin skeletons was studied by electron microscopy. It was found that an electron-dense layer was located im-

mediately inside the S_1 layer. This region was more highly lignified than any other portion of the cell wall but also contained cellulose microfibrils which were considered to be oriented in a direction transverse to the fiber axis. The inner part of S_2 had a uniform distribution of lignin in the form of a continuous network.

It is evident that controversies exist concerning the distribution of lignin in compression wood. One of the objectives of the present investigation was to adduce additional information and to compare normal and compression wood in this respect. Other objectives were to study the distribution of lignin in the horizontal ray cells and the orientation of the microfibrils in the S_2 layer of compression wood tracheids. The species chosen was tamarack [*Larix laricina* (Du Roi) K. Koch] because it had been found that the polysaccharides could be more readily removed from compression wood of this species than had been the case with either the spruce or the fir used previously [TIMELL, CÔTÉ, and ZABEL 1966].

Materials and Methods

Wood of tamarack was collected from trees located in a bog in the neighborhood of Meacham Lake, Adirondack Mountains, New York. All had grown for a considerable time in a horizontal position with the top eventually attaining a vertical position; wood was collected only from the horizontal portions. Cross-sections of the stems exhibited extreme eccentricity, as has been shown elsewhere [CÔTÉ, DAY, SIMSON and TIMELL 1966]. Compression wood was taken from sapwood within the last few growth rings. Normal wood of similar age was obtained from vertically growing trees.

Squares of wood, 8 mm × 8 mm, with a thickness of 100 μm were cut with a microtome and extracted in the usual way with ethanol-benzene. Polysaccharides were removed by the method of SACHS, CLARK and PEW [1963], albeit with some modifications. Several specimens were immersed in 30% aqueous hydrofluoric acid at room temperature (20 ... 25° C) in a closed polyethylene bottle and kept there for three hours. The concentration was increased gradually in increments of 10% until a final concentration of 80% had been reached. The sections were kept in this acid for seven days at room temperature, after which the concentration was reduced stepwise to 30%. For removal of the last traces of polysaccharides, the sections were treated with 4% aqueous hydrochloric acid at 98° C for six hours. Acid was removed by careful decantation with distilled water. The specimens were stored in water at 4° C. If allowed to dry, the lignin skeletons had a tendency to collapse, giving a coherent material, not reflecting the original biostructure [COWLING 1965].

For elimination of lignin, small cubes of various sizes were immersed in aqueous solutions containing 10 g of sodium chlorite and 5 ml of glacial acetic acid per 100 ml solution. Oxidation was carried out at 50° C for times varying from 3 ... 72 hrs. Delignification was complete after 12 hrs. The sections had a tendency to disintegrate after 48 hrs. Salts and acid were removed by frequent decantation with water, after which the holocelluloses were stored in absolute ethanol.

Specimen preparation and electron microscopy were carried out as described previously [CÔTÉ, TIMELL and ZABEL 1966]. The embedding medium (methacrylate) was removed from all sections before shadowing.

Results

Normal and compression woods contained 27% and 38% lignin, respectively; other analytical data have been reported elsewhere [CÔTÉ, SIMSON and TIMELL 1966]. A light micrograph of a cross-section of tamarack compression wood is seen in Fig. 1, showing the rounded outline of the tracheids, the intercellular spaces, and the cavities in the secondary wall. Also visible is a thin, dark band inside the S_1 layer. An electron micrograph of a springwood section is shown in Fig. 2. It is evident how small is the proportion of the total volume that is occupied by the compound middle lamella, partly because of the numerous, large intercellular openings and partly because of the thin layer of intercellular material.

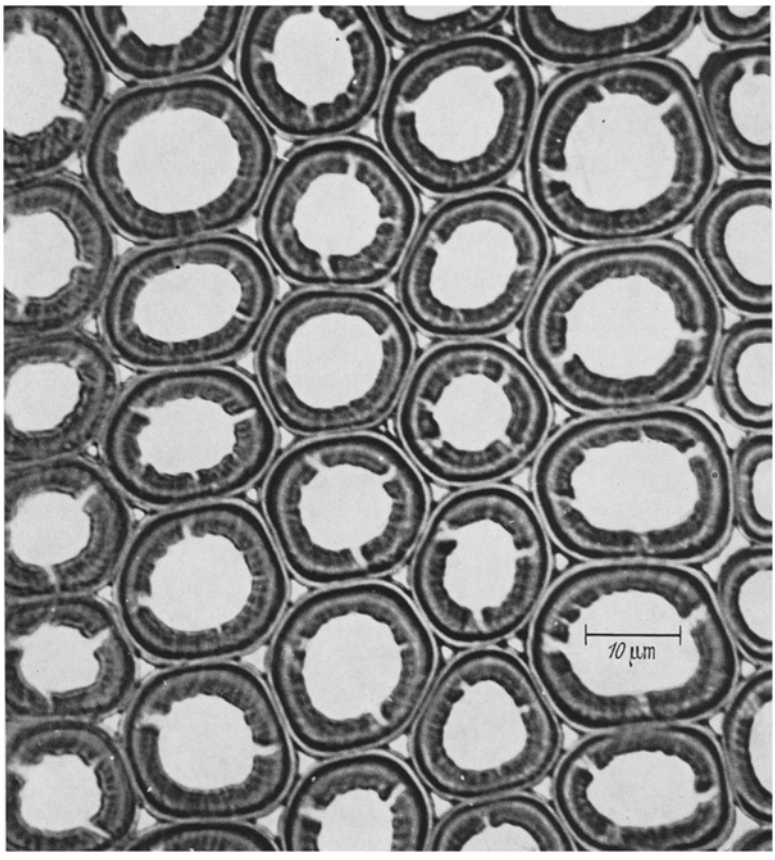

Fig. 1. Transverse section of compression wood of *Larix laricina*. Light micrograph.

The first, fairly thick and clearly lamellated cell wall layer is S_1. Following this is a coherent, somewhat darker region of approximately the same thickness, representing the outer part of S_2. The inner portion of S_2 contains the helical cavities. It should be noted that the latter all end at approximately the same distance from the lumen. Fig. 3 shows tracheids from the same growth ring at the springwood-summerwood boundary. The cells are more elliptical or flattened in the latewood, and the narrow, last-formed tracheid contains fewer cavities than do the other three. Faint traces of the primary walls can be seen here

adjacent to the S_1 layer which is thickened at the cell corners and clearly lamellated. The two first-formed springwood cells have a square outline and appear to lack helical cavities, as has been discussed in detail by CÔTÉ, DAY, KUTSCHA and TIMELL [1968].

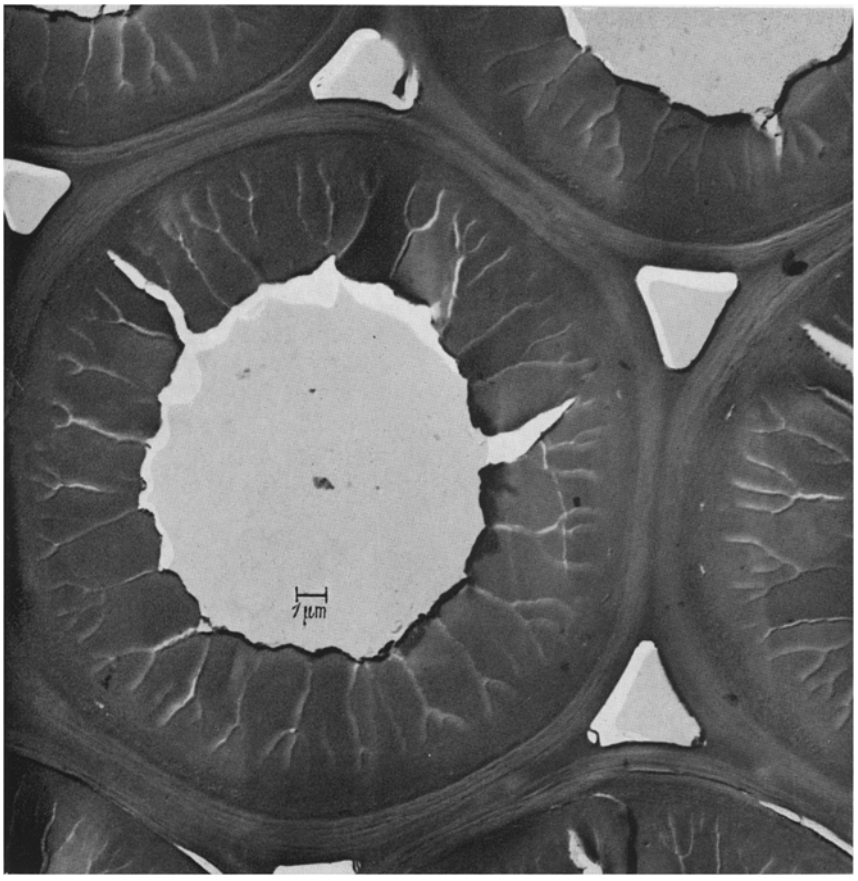

Fig. 2. Transverse section of compression wood in the springwood area. Electron micrograph.

A transverse section of three adjacent tracheids in normal tamarack wood, from which all polysaccharides had been eliminated by treatment with aqueous hydrofluoric acid, is shown in Fig. 4. The dense, isotropic lignin in the region of the former middle lamella and the lignin meshwork in the former secondary wall are similar to those observed previously by MÜHLETHALER [1949], MEIER [1955], JAYME and FENGEL [1961], SACHS, CLARK, and PEW [1963], and CÔTÉ, TIMELL and ZABEL [1966]. In the former S_1 layer, the lignin appears to be oriented in a transverse direction to the fiber axis. The region bordering on the lumen (S_3) in this micrograph appears to contain more lignin than the remainder of the secondary wall.

No such phenomenon is noticeable in Fig. 5, where the lignin density is uniform across the entire secondary wall. A transverse orientation of the lignin is evident

in both S_1 and S_3, the latter layer being considerably thinner than the former. Fig. 6 is a tangential section through the same material. The openings in the S_1 layer left on removal of the cellulose microfibrils indicate that the latter were running at a very steep angle to the fiber axis. In S_2 the lignin appears to be laterally oriented, as could be expected. The orientation of the lignin in the former S_3 layer, which is clearly outlined, indicates that the section was somewhat oblique.

The same features can be seen in Fig. 7, which is a tangential section; also shown are parts of two ray tracheids in cross section. It has long been known that the rays in conifers are more highly lignified than are the tracheids [HARLOW and WISE 1928; BAILEY 1936] and this is clear from the micrograph, which shows a very dense lignin network, uniformly distributed throughout the secondary wall of the ray cells.

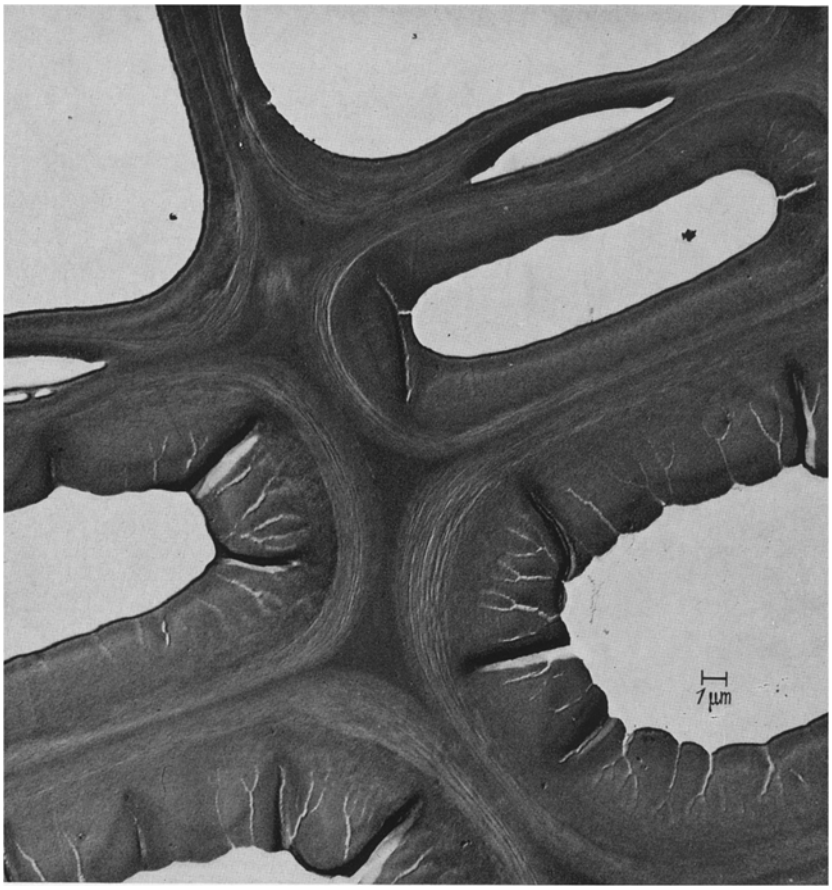

Fig. 3. Transverse section of compression wood at the summerwood-springwood boundary, showing parts of four summerwood and two first-formed springwood tracheids.

A transverse section of compression wood tracheids which had been treated with hydrofluoric acid is shown in Fig. 8. The proportion of lignin present in the compound middle lamella is very slight, primarily because of the numerous and large intercellular spaces. The small quantity of lignin occurring in the former S_1

layer is oriented in a direction transverse to the fiber axis. Immediately inside S_1 there is a layer of approximately the same thickness which appears to be lignified to the same extent as the compound middle lamella. The helical cavities extend up to, but do not enter, this region. The remainder of S_2 contains a dense and uniform lignin network.

The lateral organization of the lignin in the former S_1 layer is clearly shown in Fig. 9. Faint traces of the former primary wall can also be seen here. Fig. 10, which includes an unusually large intercellular space, reveals how closely these openings can approach the secondary wall. In this case, the lignin barely encrusts the primary wall.

With a view of gaining additional insight into the distribution of lignin, small cubes of compression wood were treated with chlorous acid for various lengths of time. As can be seen from Fig. 11, the compound middle lamella, the S_1 layer and, especially, the outer region of S_2 were attacked first, while the inner part of S_2 appears relatively unchanged at this stage. Although interpreted differently, these are observations similar to those made previously by CASPERSON [1963].

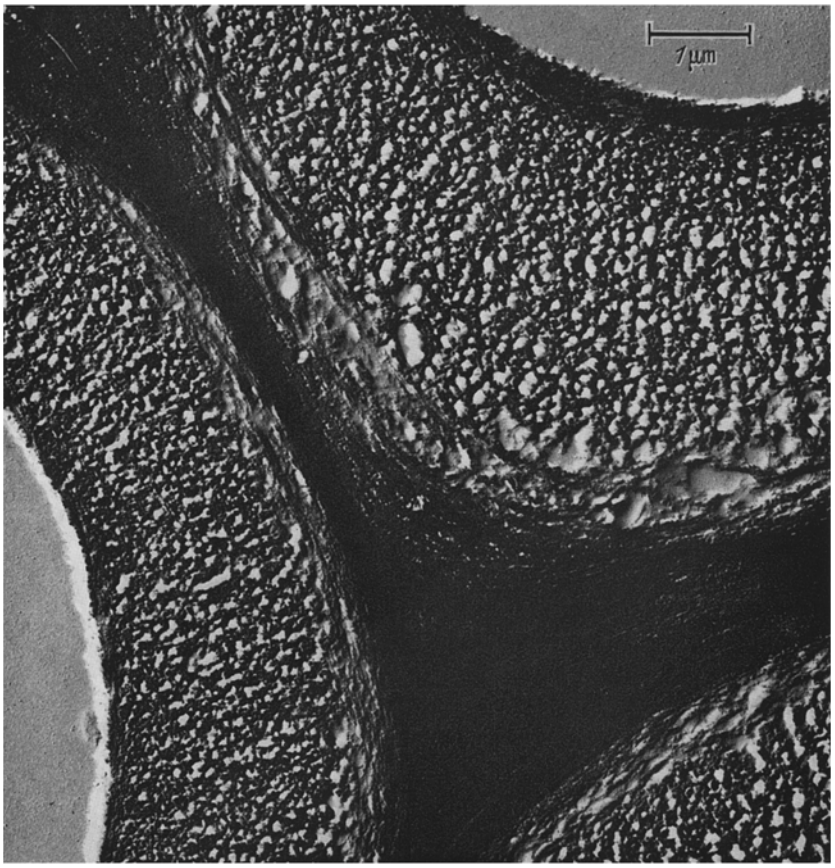

Fig. 4. Transverse section of three tracheids of normal wood treated with aqueous hydrofluoric acid to give a residual lignin skeleton.

Fig. 12 shows a transverse section of a compression wood tracheid from which all lignin had been eliminated. With normal wood, such sections always exhibit excessive swelling or disintegrate completely; with compression wood this is seldom observed. Some of the primary wall has been lost, but remnants can be seen in several places. The lamellar nature of the former massive S_1 layer is immediately evident, and it is clear that not much lignin could have been present in this region. The outer portion of S_2 contains a very loose meshwork of cellulose

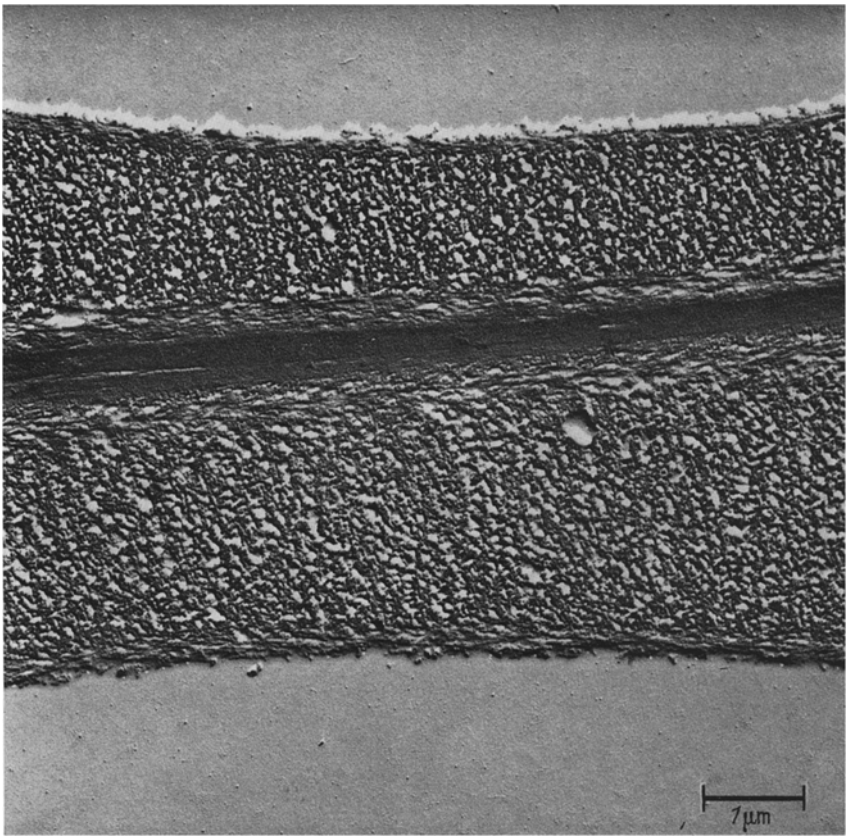

Fig. 5. Transverse portion of two tracheids of normal wood. Lignin skeleton. Note the uniform distribution of the lignin and its transverse orientation in the S_1 and S_3 layers.

microfibrils, while the inner zone, where the cavities are located, consists of denser lamellae of cellulose. The low carbohydrate concentration in the outer region of S_2 is seen even more clearly in Fig. 13. Outside the former S_1 layer loosely organized microfibrils from the primary wall can be detected, and individual cellulose microfibrils can be seen in the former, lignin-rich layer inside S_1. The texture of these microfibrils indicates that they probably had the same orientation in the original tracheid as had the microfibrils in the inner S_2 layer, that is, approximately 45° to the fiber axis.

Fig. 14 shows a longitudinal section through the same tracheid. In the former S_1 layer there are now spaces left by the lignin, another indication how this con-

stituent follows the orientation of the microfibrils. The texture of the outer and inner portions of the former S_2 layer is the same as that observed in transverse section (Fig. 13), suggesting that the microfibrils very likely are oriented at an angle of 45° to the fiber axis.

A tangential section through compression wood, which had been treated with hydrofluoric acid, is shown in Fig. 15, including cross sections of three ray cells. The less highly lignified S_1, the lignin-rich region inside this layer, and the helical cavities can be seen in the tracheids on each side of the ray cells. The latter appear to be lignified to approximately the same extent as the inner part of S_2 in the tracheids. This is confirmed by the micrograph in Fig. 16, which also shows the

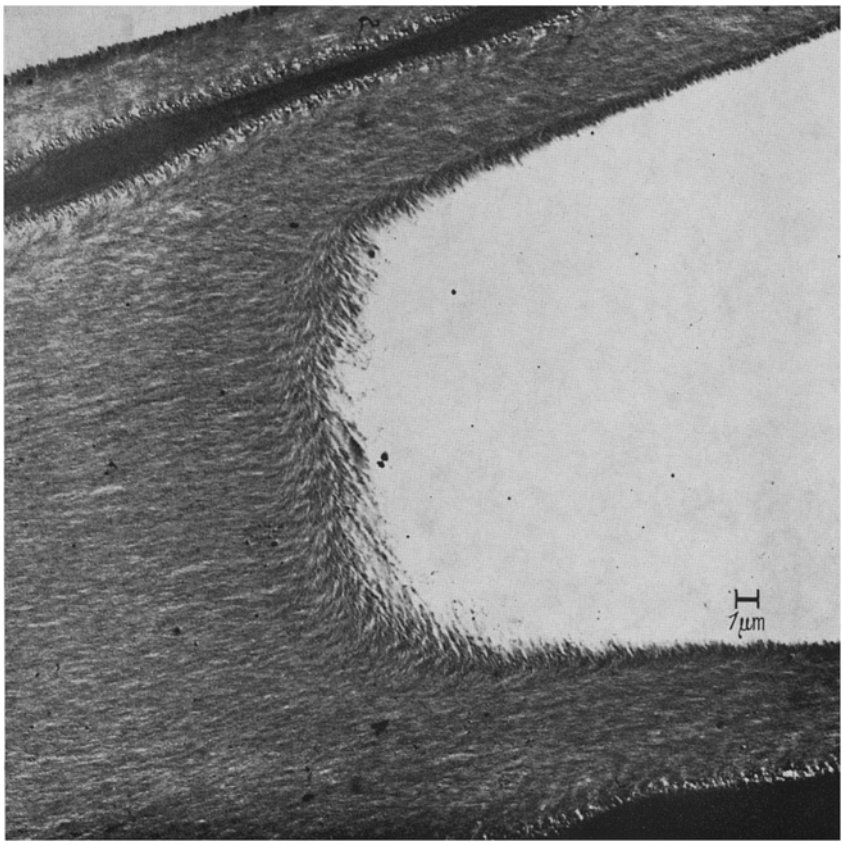

Fig. 6. Tangential section of portions of two tracheids of normal wood. Lignin skeleton. Note the longitudinal orientation of the lignin in the former S_2 layer and the different appearance of the S_1 and S_3 layers.

lamellar nature of the laterally oriented lignin, a texture quite different from the meshwork observed in transverse sections of tracheids from normal wood (Fig. 4).

An oblique, radial section of a compression wood tracheid was partly delignified with acid chlorite and carbon replicas were prepared. An example is shown in Fig. 17. It is readily seen that the cellulose microfibrils in the helical ribs are oriented in the same direction as the latter. It is also clear that both the size of

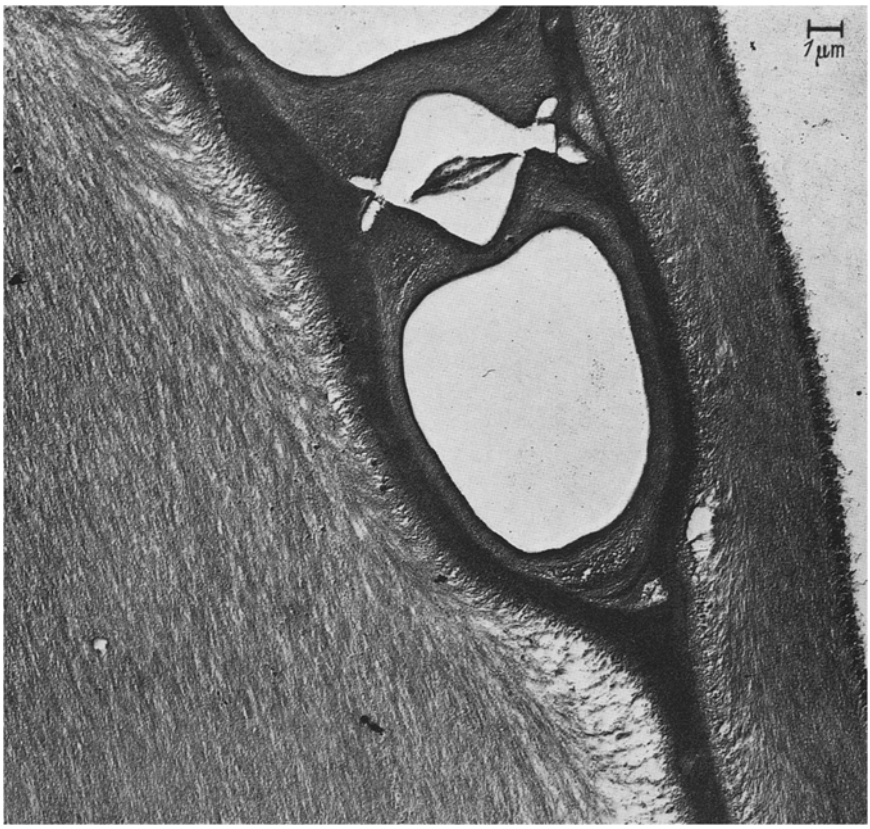

Fig. 7. Tangential section of lignin skeleton of normal wood, showing two ray cells in cross section. Note the dense lignin network in the ray cells.

the ribs and the distance between them show considerable variations. The cell wall to which they are attached can be seen between the ribs. The major orientation of the cellulose microfibrils in this wall is the same as that of the microfibrils in the ribs. In the area at the lower right of the micrograph there are widely spaced, parallel microfibrils embedded in a matrix of amorphous material, probably lignin, showing the outer region of the S_2 layer to which the helical ribs are attached.

In a previous study [CÔTÉ, DAY, KUTSCHA and TIMELL 1968] it was found that many conifer species lack some of the typical compression wood features in their first-formed springwood tracheids but that the orientation of the microfibrils in S_2 probably is the same as that in later developed cells. A section similar to that in Fig. 17 is shown in Fig. 18 for the first tracheid formed at the beginning of the growing season. Helical cavities are definitely present in this case but are less well developed than in later-formed cells. The microfibrils run parallel to the direction of the cavities, both in the ribs themselves and in the cell wall to which the latter are attached, thus indicating the correctness of the earlier conclusions.

Discussion

The fact, recently discussed by Berlyn and Mark [1965], that the larger part of the lignin in a softwood is present in the secondary wall and not in the compound middle lamella, as was formerly believed, is clear from the lignin skeletons in Figs. 4, 5, and 6. A quantitative evaluation of the present experimental material would offer considerable difficulties. Goring [unpublished], using ultraviolet microscopy, has recently estimated that the compound middle lamella probably consists of 60% of lignin, a value in agreement with that of Bailey [1936] who, on direct analysis of this region found it to contain 70% lignin. Recently, Fergus, Procter and Goring [unpublished] have estimated that the compound middle lamella between the cell corners contains approximately 50% and at the cell corners about 80% lignin. They have also found that approximately 25% of the total lignin is located in the compound middle lamella. Previous statements, dating back to the pioneering studies of Ritter [1925] and automatically repeated in the literature for forty years afterwards, must be ascribed to

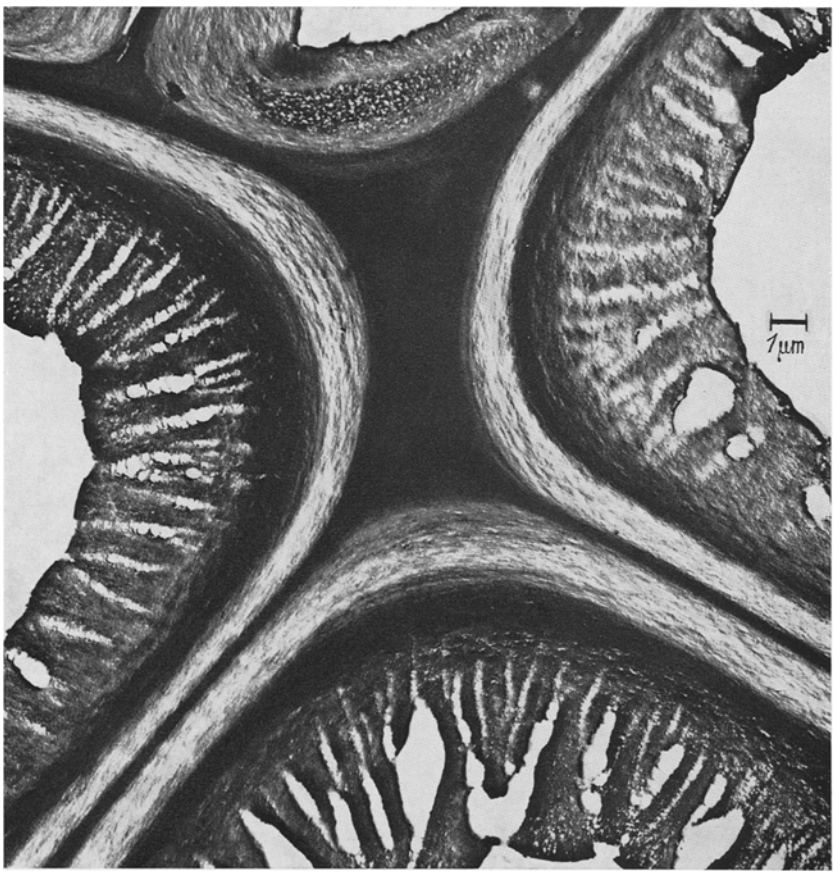

Fig. 8. Transverse section of compression wood tracheids treated with hydrofluoric acid to give a residual lignin skeleton. Note the low concentration and transverse orientation of the lignin in the thick S_1 layer, the dense layer of lignin in the outer portion of S_2, and the high lignin concentration in the inner part of S_2.

sheer oversight, since available evidence, for example that reported by LANGE [1954], if properly evaluated, gives the values computed by BERLYN and MARK [1965].

LANGE [1945] noted that wood exhibits dichroism in ultraviolet light, and he interpreted this in terms of an oriented adsorption of the lignin macromolecules onto the cellulose microfibrils. This view has been criticised by RUCH and HENGARTNER [1960] and by FREY-WYSSLING [1964], who have pointed out that this is merely from anisotropy, and that lignin is actually completely amorphous.

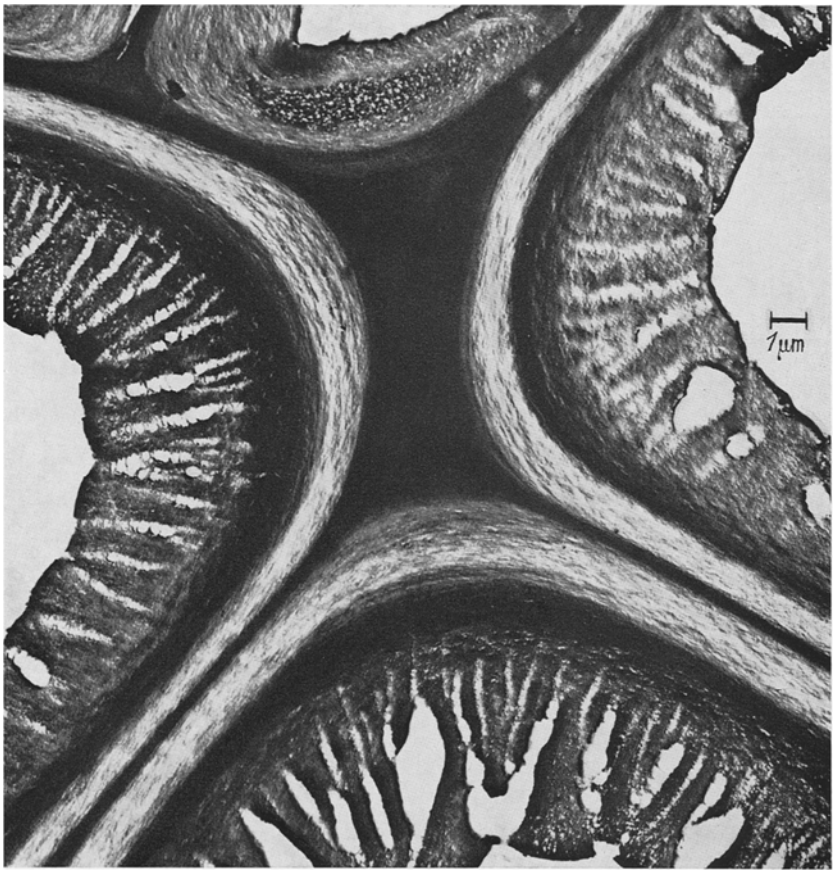

Fig. 9. Transverse section of lignin skeleton of compression wood tracheids, showing trace outline of the former primary wall outside S_1.

No orientation of lignin occurs *on the molecular level.* In wood, the microfibrils of cellulose are probably embedded in a matrix of hemicelluloses [MEIER 1965]. LIANG, BASSETT, MCGINNES, and MARCHESSAULT [1960], using polarized infrared techniques with thin sections of wood, have shown that the major wood hemicelluloses, albeit undoubtedly amorphous, are oriented to a certain extent in the direction of the cellulose microfibrils. The same situation would be expected to apply to lignin, as pointed out by JAYME and FENGEL [1961], who noted such an orientation of the lignin in the S_1 layer.

Inspection of the micrograph in Fig. 5 reveals that the lignin follows the transverse orientation of the cellulose microfibrils, not only in the S_1 but also in the S_3 layer. All three layers of the secondary wall are known to consist of lamellae of microfibrils. These lamellae can readily be traced in the S_1 and S_3 layers in the transverse section of Fig. 5, and they can also be seen in S_2 in the longitudinal section in Fig. 6. All three layers of the secondary wall of a softwood are accordingly similar in this respect. The fact that lignin aligns itself so closely with the cellulose microfibrils is not surprising considering that the well-ordered microfibrils are deposited in the various cell wall layers *before* becoming encrusted with the amorphous, three-dimensional lignin.

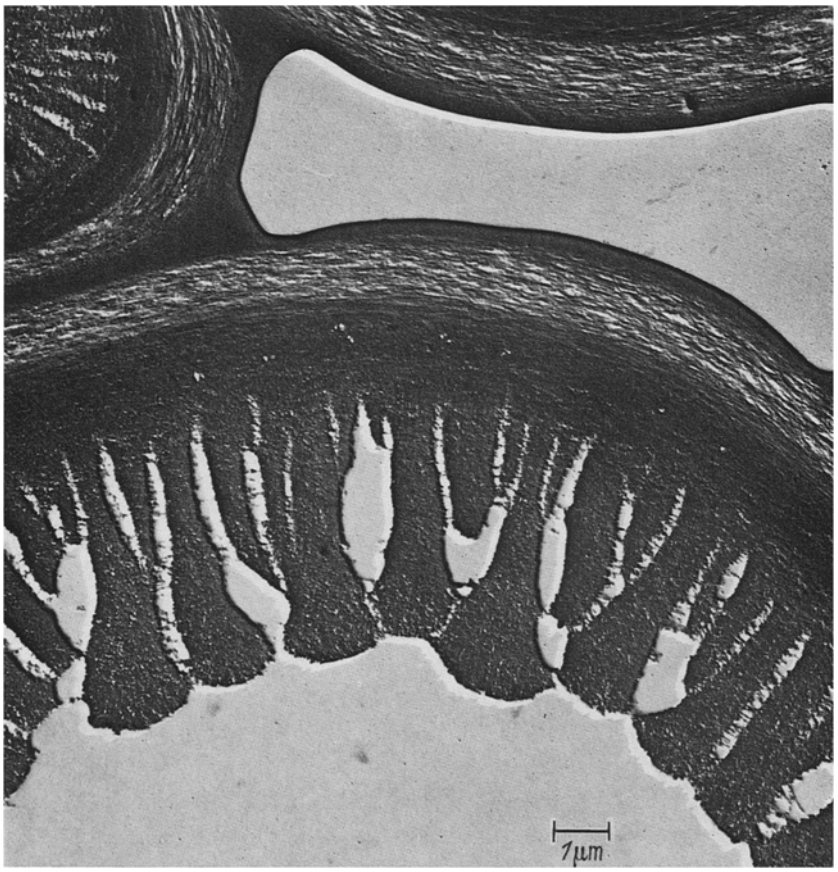

Fig. 10. Transverse section of lignin skeleton of compression wood tracheids, showing unusually large intercellular opening, extending to the former primary wall.

The holes of various sizes in S_2 which are seen in the transverse sections in Figs. 4 and 5 represent areas where the longitudinally oriented cellulose microfibrils were located prior to their removal. These openings do not necessarily reflect the actual size of these microfibrils since they have probably become enlarged by the swelling that always takes place when cellulose is treated with acids. The texture of the lignin network in S_2 also seems to vary with the acid used. Jayme and Fengel [1961], for example, noted that the lignin skeletons observed

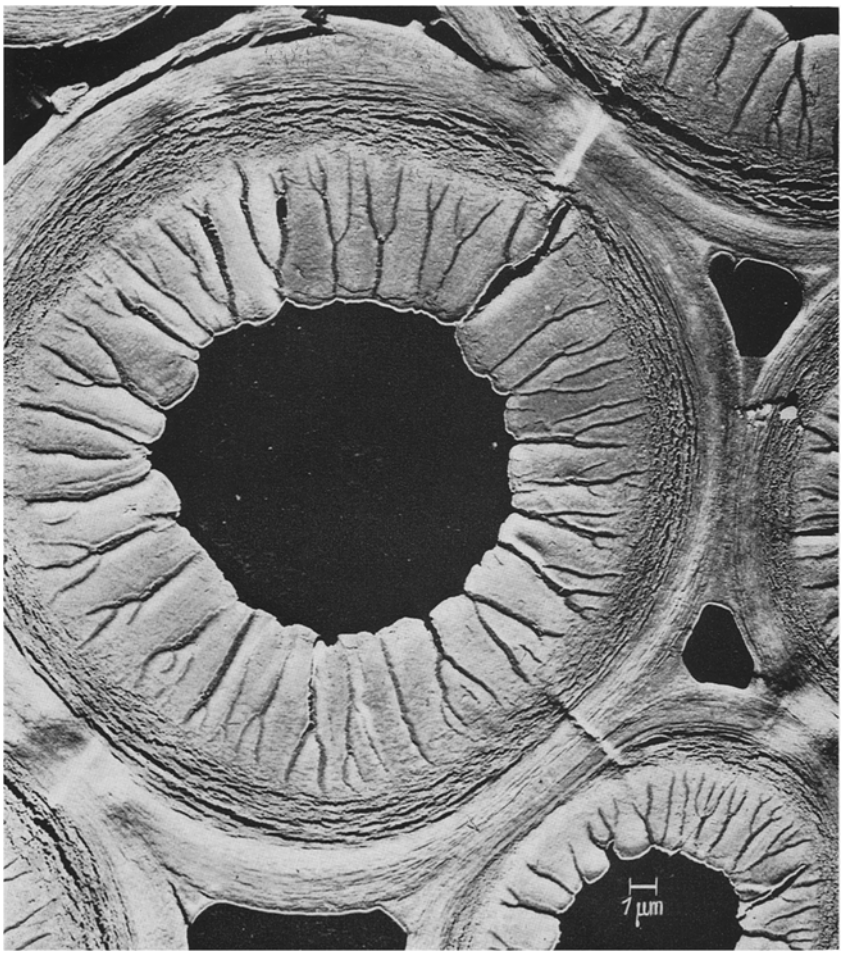

Fig. 11. Transverse section of compression wood tracheids at an early stage of delignification with chlorous acid. Middle lamella at lower left has been removed. Lignin in outer part of S_2 has been partly eliminated, revealing the lamellar nature of this layer.

on treatment of thin softwood sections with a mixture of sulfuric acid and phosphoric acid are considerably denser than those obtained with hydrochloric acid.

The S_3 layer in softwoods, which appears to contain a high proportion of arabinoglucuronoxylan [MEIER and YLLNER 1956; MEIER and WILKIE 1959] has also been claimed to contain more lignin than the remainder of the secondary wall. According to JAYME and FENGEL [1961] S_3 is more highly lignified than S_2 in tracheids of *Picea abies*. SACHS, CLARK and PEW [1963] state that in *Picea glauca* and *Pinus taeda* the lignin in S_3 is almost as dense as that in the compound middle lamella. CÔTÉ, TIMELL and ZABEL [1966] oberserved a denser lignin network in the regions closest to the lumen of tracheids from *Picea rubens*. SACHS [1965] has reported that in *Fagus sylvatica*, which has been subjected to either natural or chemical decay, the greatest densities of lignin appear in the compound middle lamella and in S_3.

Recently, FENGEL [1965] has drawn attention to the fact that, when polysaccharides are removed from wood by hydrolysis with acid, the remaining material will not necessarily consist only of lignin but represents any cell wall constituents not amenable to hydrolysis. This is a viewpoint which is especially pertinent when studying the degree of lignification of S_3, since this layer is usually followed by a warty layer, which cannot be completely hydrolyzed. The higher

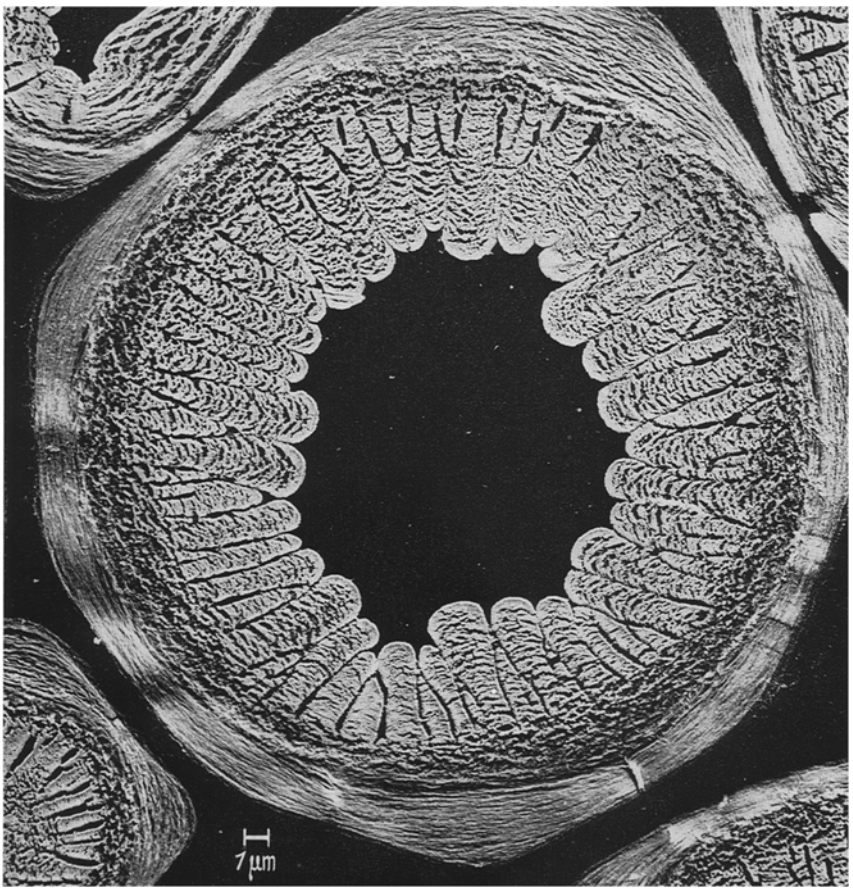

Fig. 12. Transverse section of compression wood tracheid, completely delignified with chlorous acid. Note the dense lamellae of microfibrils in S_1, the thin network in the outer portion of S_2, and the denser lamellae of cellulose in the inner part of S_2. Remnants of the primary wall are seen outside S_1.

lignin density often observed for S_3 appears to be somewhat erratic. It can be observed in the micrographs of JAYME and FENGEL [1961] only in those cases where sulfuric acid-phosphoric acid was used, but not when hydrolysis of the polysaccharides was effected with hydrochloric acid. In the investigation of CLARK, SACHS and PEW [1963], a high density can be observed in S_3 in sections of *Picea glauca* hydrolyzed with hydrofluoric acid or treated with p-(acetoxymercuri) aniline. There seems to be no increase in lignin concentration towards the lumen when the polysaccharides were removed either chemically with periodic acid or enzymatically by treatment with *Trichoderma viride*. It is clear from this

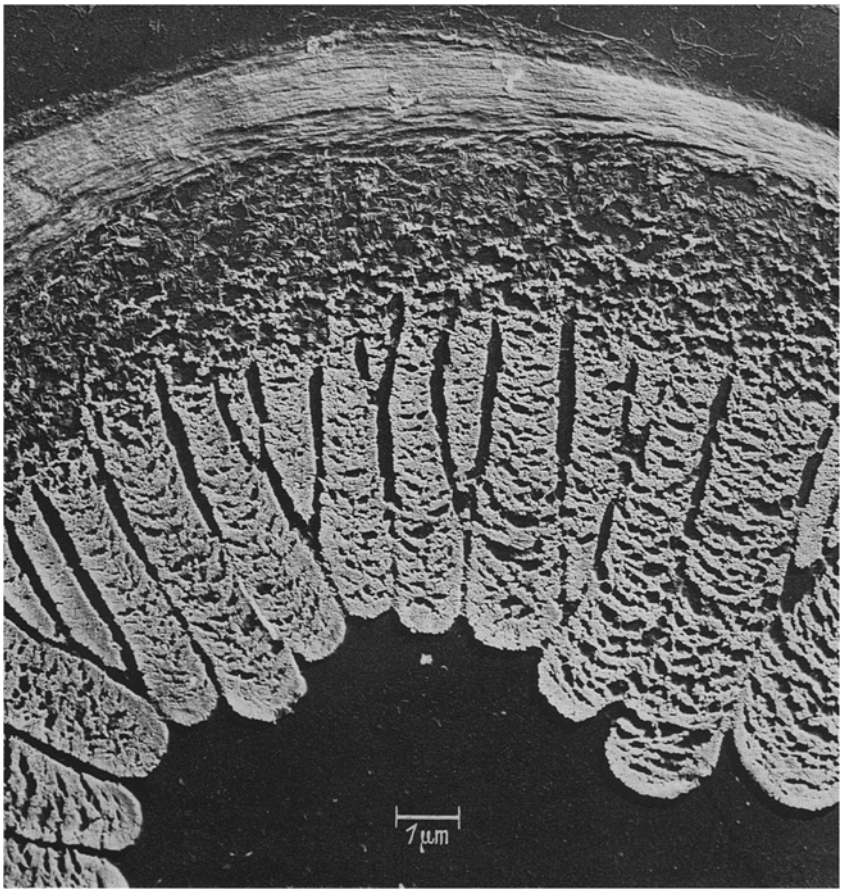

Fig. 13. Transverse section of delignified compression wood tracheid, showing primary wall, S_1, loose network of microfibrils in outer S_2, and inner S_2. Individual microfibrils can be seen in outer S_2.

study that the warty layer in untreated sections of *Pinus taeda* has a very high electron density, and the results of CÔTÉ, TIMELL and ZABEL [1966] indicate that this layer remains after treatment with hydrofluoric acid.

In the present investigation, some of the electron micrographs of lignin skeletons, such as that shown in Fig. 4, suggested a higher lignin concentration in the neighborhood of the lumen, while others, exemplified by Figs. 5 and 6 indicated a uniform lignin density across the secondary wall. It is our opinion that these two micrographs represent the true lignin distribution, and that in softwoods neither S_1 nor S_3 contain more lignin than S_2. As already emphasized by FREY-WYSSLING [1964], lignification is accordingly uniform throughout the entire secondary wall.

In accordance with earlier analytical data, ray cells in normal wood of tamarack were found to be highly lignified. FENGEL [1965], who used the same techniques for studying the lignin distribution in ray cells of *Fagus sylvatica*, found these to contain a very open and loose network of lignin in the secondary wall. No distinct

cell wall layers can be detected in the present case (Fig. 7), but this matter will have to await further investigations.

The viewpoints of Casperson [1959, 1963, 1965], Casperson and Zinsser [1965], Correns [1961], Wergin [1965], and Wergin and Casperson [1961], concerning the ultrastructure of the tracheid cell wall in compression wood are not in agreement with our own, nor do they agree with those of Bailey and Berkley [1942] or Davies and Wardrop [1964]. The major difference resides

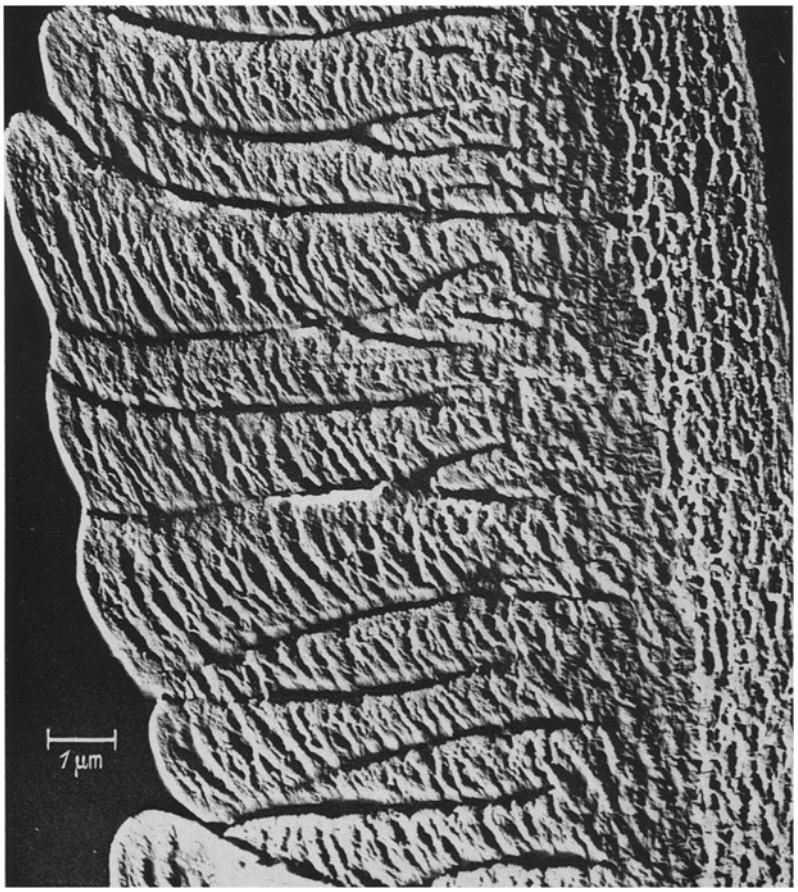

Fig. 14. Longitudinal section of delignified compression wood tracheid.

in the identification of the primary wall, S_1, and the outer, lignin-rich layer of S_2. What Casperson, Correns and Wergin consider to be a "thick primary wall", we regard as the S_1 layer, and their "thin S_1 layer" we hold to be the outer, lignin-rich region of the S_2 layer. Because of this basic difference in interpretation of ultrastructure, any comparison between the distribution of lignin found in the present study and that reported for similar material by Wergin [1965] would be of little value. Our own data show that, contrary to the statements of the above investigators, the S_1 layer in compression wood tracheids is considerably thicker than the corresponding region in normal wood. Direct measurements [Côté, Kut-

SCHA, SIMSON and TIMELL 1968] have given a ratio between $M + P$, S_1 and S_2, of 1 : 8 : 41 for normal and 1 : 12 : 37 for compression wood tracheids of *Abies balsamea*. It is also evident that the helical cavities do not extend up to the primary wall, as stated by CORRENS [1961] but terminate inside S_2, nor is the primary wall thicker in compression than it is in normal wood.

The proportion of lignin located in the compound middle lamella is extremely variable because of the fluctuating frequency of the intercellular openings. In a section, such as that shown in Fig. 8, probably only 5 ... 10% of the total lignin is extracellular. This situation is responsible for the fact that compression wood, in spite of its high lignin content, is no more difficult to defibrate in pulping than is normal wood [DADSWELL and WATSON 1957; DADSWELL, WARDROP and WATSON 1958].

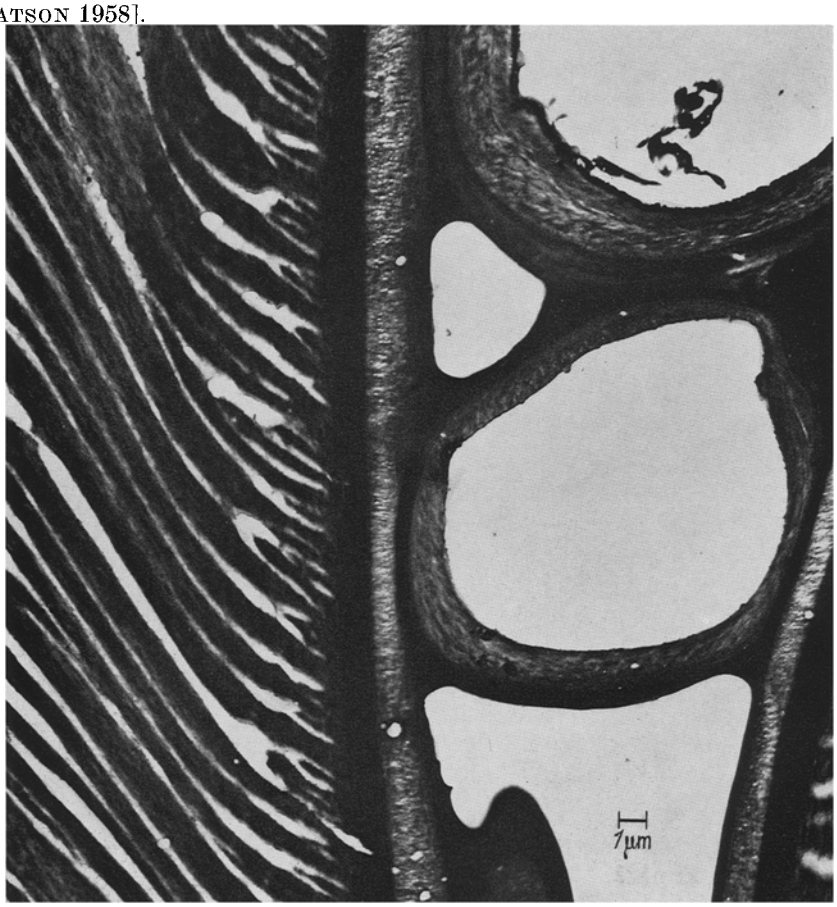

Fig. 15. Tangential section of compression wood treated with hydrofluoric acid, showing cross section of three ray cells, which seem to be lignified to the same extent as the inner part of the S_2 layer in the tracheid.

As first shown by BAILEY and BERKLEY [1942] and later confirmed by WARDROP and DADSWELL [1950], the orientation of the microfibrils in the S_1 layer of compression wood tracheids is almost transverse to the fiber axis. Our results show (Fig. 13) that the polysaccharide density is very high in this layer and that

the microfibrils are organized in lamellae. The concentration of lignin in this region is very low; the small amounts of lignin present follow the orientation of the cellulose microfibrils (Figs. 8 and 9).

Immediately inside S_1 there occurs a region with a very high concentration of lignin, as first noted by BAILEY and BERKLEY [1942]. This layer probably has the same concentration of lignin as has the compound middle lamella, that

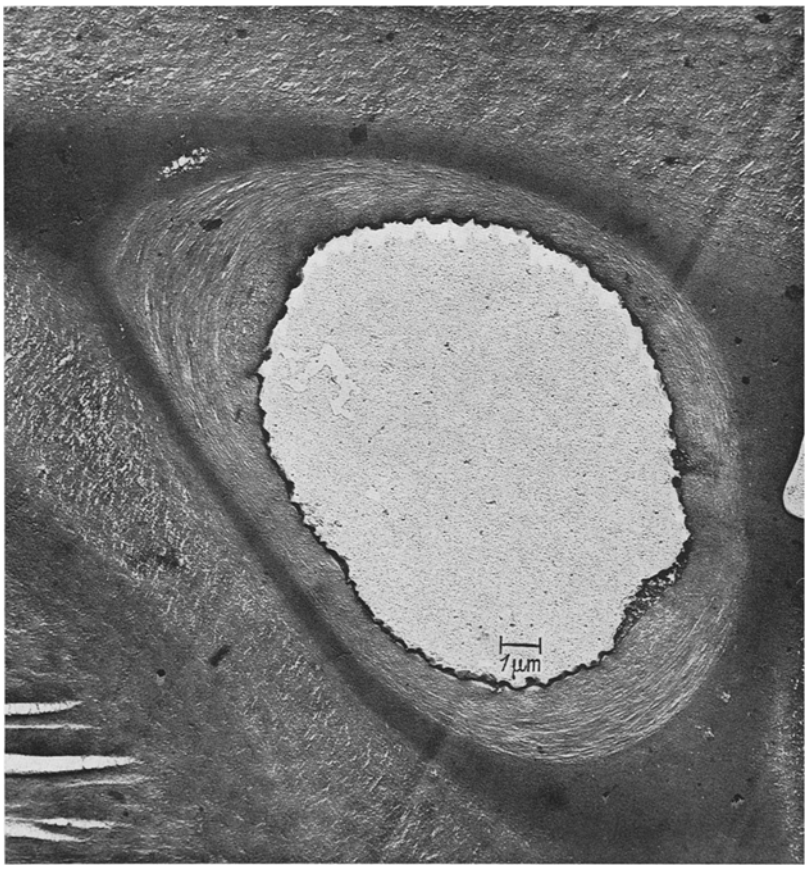

Fig. 16. Transverse section of compression wood treated with hydrofluoric acid, showing a ray cell in cross section. Note lamellar nature of the lignin in the ray cell.

is, 60 ... 70%. Studies on developing compression wood tracheids [CASPERSON and ZINSSER 1965; CÔTÉ, KUTSCHA and TIMELL, unpublished] show that lamellae of cellulose occur in this region. This is also evident from the holocellulose micrographs in Figs. 12, 13, and 14, which also indicate that the polysaccharide concentration must be quite low, as was originally predicted by WARDROP and DAVIES [1964]. The orientation of these microfibrils is probably the same as in the remainder of S_2. The transverse orientation suggested by CÔTÉ, TIMELL and ZABEL [1966] could not be confirmed.

The helical cavities or ribs, which run through the inner part of S_2, all terminate when they reach the lignin-rich layer. As was shown by WERGIN and CASPERSON [1961] and by CASPERSON [1963], the inner part of S_2 contains lamellae of cellu-

lose (Figs. 12, 13 and 14). It also has a high concentration of lignin which is uniformly distributed in a dense network. The statement of LANGE [1954] that this region should be free of lignin is not correct.

The horizontal ray cells in compression wood are usually assumed to be similar to those in normal softwoods. They were found here (Figs. 15 and 16) to be lignified to approximately the same extent in the two types of wood. In comparison with the respective tracheids, however, the ray cells in compression wood are less highly lignified than are those in normal wood. The lignin is uniformly distributed and is lamellated across the entire secondary wall. A warty layer is present adjacent to the lumen.

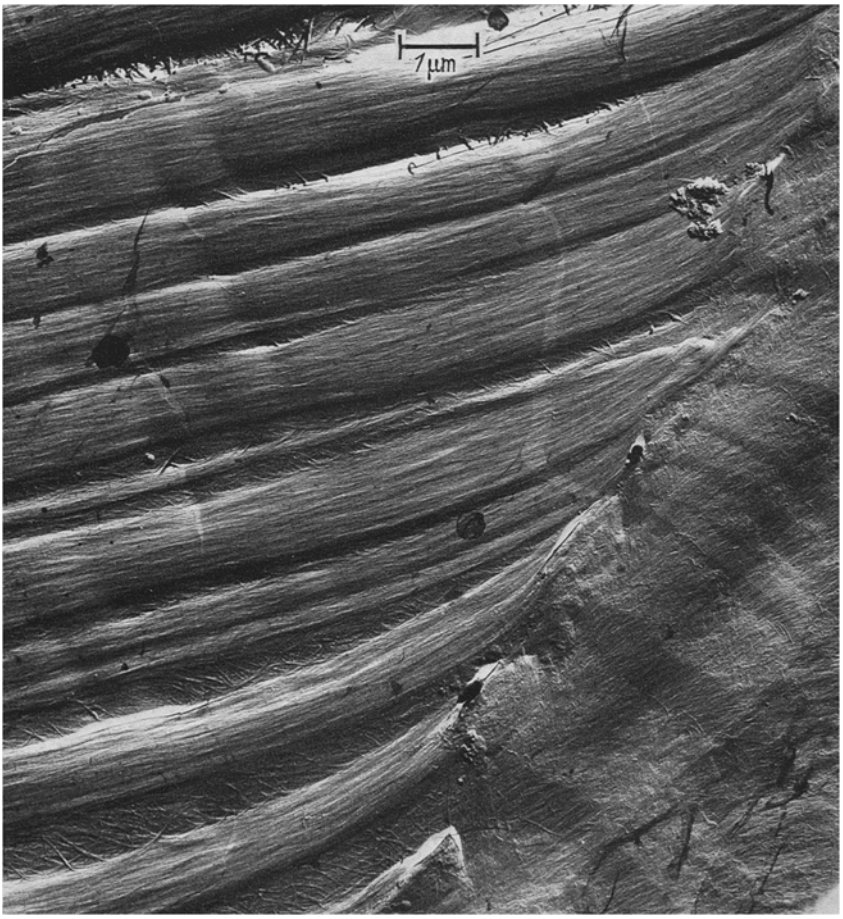

Fig. 17. Oblique (15°), radial section of fully developed compression wood tracheid, partly delignified with chlorous acid. Note orientation of microfibrils in the ribs and in the cell wall to which the ribs are attached.

Conclusions

The distribution of lignin in compression wood tracheids is clearly different from that in tracheids of normal wood, the major difference being the occurrence of a fairly thick, lignin-rich layer in the outer part of S_2, immediately inside S_1.

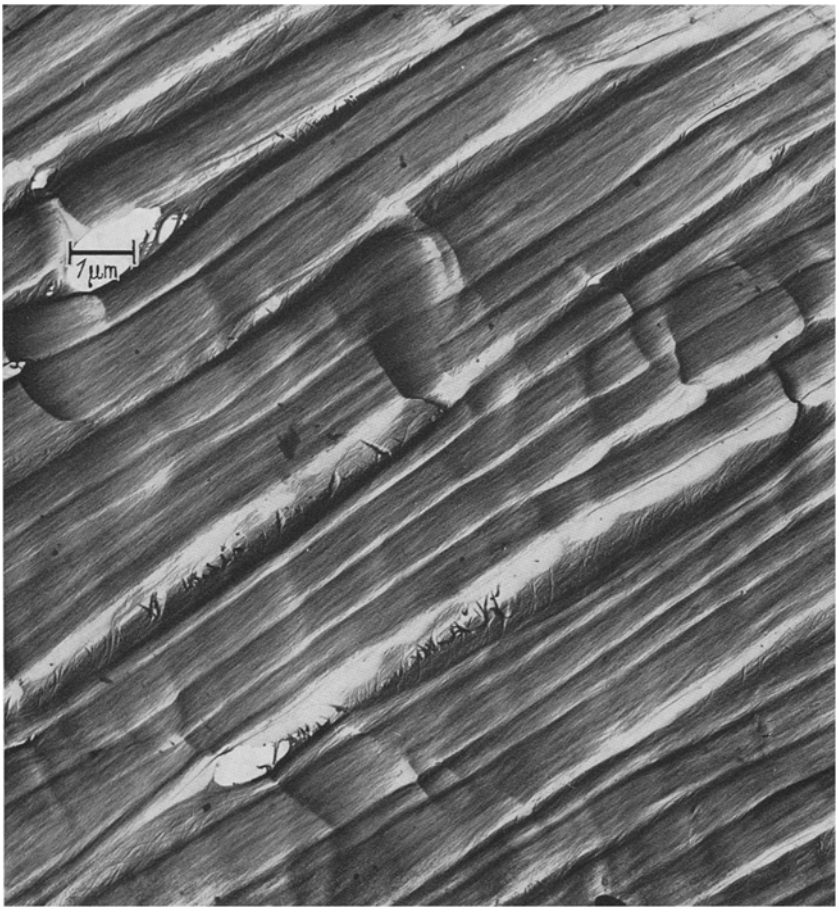

Fig. 18. Oblique (15°), radial section of first-formed compression wood tracheid in the springwood area. Lignin partly removed with chlorous acid. Note orientation of the microfibrils in the helical ribs which are only partly developed.

The S_1 layer is also less highly lignified than in normal wood, where all three layers in the secondary wall are lignified to approximately the same extent. This is in contrast to the situation in hardwoods, where S_1 shows a higher lignin concentration than does S_2 [CÔTÉ, BENTUM, DAY and TIMELL, unpublished].

The outer part of S_2 in compression wood tracheids is quite distinct from the inner portion in its lack of helical cavities, and in its low polysaccharide and high lignin contents. That it must nevertheless be deemed a part of S_2 is evident from the fact that the cellulose microfibrils have the same orientation in the two regions. For ease of reference, it would perhaps be useful in the future to refer to this layer as $S_2(L)$ (where L would signify lignin), just as the gelatinous layer in tension wood now is often referred to as $S_2(G)$, $S_3(G)$ or $S_4(G)$ [WARDROP 1964]. Perhaps also, in this way, controversy concerning the nature of the different cell wall layers in compression wood tracheids could be avoided.

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