CHEMICAL AND MORPHOLOGICAL ASPECTS OF THE FINE STRUCTURE OF WOOD

H. MEIER

Träkemiska avdelningen, Svenska Träforskningsinstitutet, Stockholm Ö, Sverige

In former days, wood chemists were mainly interested in obtaining information on the over-all chemical composition of trees. In recent years, however, wood anatomists, fibre morphologists, and wood chemists have started to co-operate with each other and to correlate the physical with the chemical aspects. This correlation has resulted in a much better understanding of the chemical complexity of wood.

In the present paper an outline will be given of fibre morphology and fibre chemistry as well as of the chemistry of anatomically distinct elements in wood.

The scope of the morphology of wood fibres, *i.e.* softwood tracheids and libriform fibres from hardwoods as well as other cell-elements in wood, was initially more-or-less restricted to the description of the outer shape of these cells and the arrangement of the pits. In 1926, Ambronn and Frey-Wyssling[1] showed, with the aid of the polarizing microscope, that a step forward could be taken from *microscopic* to what they called *submicroscopic* morphology. Somewhat later, X-ray methods were also applied to the investigation of the submicroscopic morphology of fibres and ten years ago electron microscopy became the ultimate tool in the field. It was then possible to see bundles of some hundred cellulose molecules, the so-called microfibrils, and it was recognized that high polymer compounds could arrange themselves into molecular aggregates peculiar to a certain molecular species.

Of the three main components in the cell walls of wood fibres, *i.e.* cellulose, hemicellulose and lignin, the cellulose has been studied most thoroughly with regard to submicroscopic structure. There has been much argument over the fine structure of the cellulose microfibrils. Mühlethaler[2], with the aid of a new electron microscopic preparation technique, the so-called " negative contrast " technique, as opposed to the shadowing technique, has now shown that the narrowest fibrils, which he calls elementary fibrils after Frey-Wyssling, have a diameter of 35 Å. These elementary fibrils, each containing 40 cellulose chains, aggregate to form larger elements, microfibrils, with a diameter which is always a multiple of 35 Å, *e.g.* 70, 105, 140 *etc.*, up to about 350 Å.

Consequently, the following cellulosic structural elements are present in the native cell wall: the cellulose molecule, the elementary fibril, the microfibril, the macrofibril, and the lamellar membrane. The macrofibrils and the lamellar membranes have no definite sizes and can be seen in the light microscope.

The submicroscopic structure of the *hemicelluloses* in wood fibres is rather obscure. Most textbooks say that the hemicelluloses are present as amorphous material between the cellulose microfibrils. However, no proof has ever been given for this statement. Some years ago, we studied the mannan hemicelluloses from dates and ivory nuts[3]. There were two mannans present, termed mannan *A* and *B*. The low molecular weight mannan *A* with a D.P. of 16 was perfectly crystalline and was present in the cell walls in the form of small granules. The high molecular weight mannan *B* with a D.P. of 80 was amorphous, but was present in the cell walls in the form of microfibrils very similar to the cellulose microfibrils in wood fibres (*Figures 1* and *2*). This indicates the following possibilities for the wood hemicelluloses: they may lie *between* the cellulosic microfibrils *either* as an amorphous or as a crystalline granular material, *or* they may form their own microfibrils which may or may not have crystalline regions. A precipitated glucomannan which we had extracted from spruce holocellulose showed no crystallinity on examination with X-rays. After mild hydrolysis, the same glucomannan gave a crystalline X-ray pattern analogous to that of ivory nut mannan *A*. This result, of course, does not shed any light on the submicroscopic structure of the glucomannan in the *native* state in the wood fibre, but it shows that if the native glucomannan molecules do crystallize, then they obviously can do so only with difficulty. If, however, their D.P. is reduced, possibly with debranching in the process, they can build up a crystalline lattice more easily. As all the hemicelluloses in wood are heteropolysaccharides, no pure mannan or pure xylan having been found, and as some are also partially acetylated, the probability that they are crystalline in the native state is not very high. Yet, they need not be completely amorphous. Most of them are only slightly branched *chain* molecules and they must, therefore, have a strong tendency to assume a parallel arrangement. In fact, Liang and co-workers[4] have recently shown, by using the polarized beam technique of infra-red spectroscopy, that the glucuronoxylan and glucomannan chains in softwood, as well as hardwood fibres, are probably arranged parallel to each other. However, the question of whether the glucuronoxylans and glucomannans form their own elementary fibrils or not has not yet been solved. In addition to ivory nut mannan, the mannans and xylans of certain algae have been found to be present in the cell walls in the form of microfibrils[5] and it is, therefore, not unlikely that the same is the case for the wood hemicelluloses, or at least for some of them.

There is not much to be said of the submicroscopic structure of lignin. Lignin differs from the polysaccharides in wood, which are almost entirely long chain molecules, in being a three dimensional macromolecule. This and the heterogeneous structures of the lignin molecules make the formation of a crystal lattice or of any other form of ordered molecular aggregate very difficult and, in fact, lignin does seem to be a completely amorphous material. The wood cells which have been studied most thoroughly with respect to fine structure are hardwood fibres and softwood tracheids. Those of most wood species are very similar. The cell walls usually consist of five layers: middle lamella (M), primary wall(P), transition layer or outer layer of the secondary wall (S_1), central layer of the secondary wall (S_2), and tertiary wall or inner layer of the secondary wall (S_3).

The two layers which are formed first in the cambium are the middle lamella and the primary wall. The middle lamella in the cambial cells is very thin but becomes thicker later when the lignin is deposited. After the delignification of transverse sections of wood, usually no microfibrils are found in the region of the middle lamella which, therefore, can be regarded as being free from cellulose.

The thin primary wall consists of an irregular network of microfibrils (*Figure 3*). In its native state this network is very loose and can easily undergo plastic deformation. The cell walls can grow and enlarge their surface as long as no other wall layers are deposited apart from the middle lamella and the primary wall. When the period of surface growth of a fibre finishes, the deposition of the secondary wall layers begins. The layer S_1, which is deposited directly on the primary wall, has also been called the transition layer because its structure is intermediate between the structures of the primary wall and the secondary wall. The microfibrils run more-or-less parallel to each other at an angle of about 50° to the fibre axis. Electron microscope studies have shown that the S_1-layer consists of two or more lamellae with a crossed fibrillar structure (*Figure 4*). The large angle which the microfibrils make with the fibre axis in the S_1-layer is responsible for its strong double refraction in transverse sections through wood, and is also of great importance in the physical behaviour of delignified wood fibres. The fibrils swell at right angles to their longitudinal axis. The S_1-layer when treated with swelling agents, therefore, shows a tendency to elongate the fibres. The S_2-layer, on the other hand, with the microfibrils running almost parallel to the fibre axis, swells at right angles to the axis and breaks open the framework of the S_1-layer, producing the well-known " balloons ". There is no doubt that the S_1-layer is a severe obstacle to the extensive swelling of fibres. In swelling agents, such as copper ethylenediamine, the swelling forces of the S_2-layer are strong enough to break open the S_1-layer. In less powerful swelling agents, *e.g.* water, the S_1-layer must be mechanically damaged before the fibres can swell without restraint.

In the S_2-layer, the so-called central layer of the secondary wall, the microfibrils are closely parallel and run in a steep helix, which forms an angle of 10 to 20° to the fibre axis (*Figure 5*). Thus, in a thin transverse wood section the S_2-layer shows only a slight double refraction in the polarizing microscope. The contact between S_1 and S_2 is very loose and in transverse sections made with a poor blade it can often be seen that S_2 has become detached from S_1. The abrupt transition between S_1 and S_2 is in striking contrast with the gradual transition between the primary wall and S_1. It is very intriguing to speculate as to why and how the cytoplasm brings about this sudden change in microfibrillar orientation in S_2.

Quantitatively, the S_2-layer is the most important layer of the fibre. Its thickness can vary from about 0·5 microns in spring wood tracheids up to about 4 microns in summer wood tracheids and is a most important factor in the stiffness of the fibres. Delignified spring wood tracheids are very flexible, while summer wood tracheids are stiff. Paper sheets which are made of summer wood tracheids only have a high tearing strength, but low tensile and bursting strength.

In the final layer of the cell wall, the tertiary wall or inner layer of the secondary wall, which borders the lumen, the microfibrils usually run in a flat spiral and, thus, this layer, like S_1, is also birefringent in transverse sections (*Figure 6*). The tertiary wall is usually rather thin, and its inner side adjacent to the cell lumen often has a surface with wart-like structures. The microfibrils are not as closely parallel as in the S_2-layer and their angle to the fibre axis can vary within wide limits as Bucher[6] and Liese[7] have shown.

Special features of the fine structure of fibres are the pits. In the tracheids of coniferous woods they are almost exclusively localized on the radial cell walls and are more numerous at the ends of the tracheids than in the middle parts. They are abundant in spring wood tracheids and relatively infrequent in summer wood tracheids, which means that the impregnation of summer wood is more difficult than impregnation of spring wood. In pine tracheids there are three different kinds of pit: the ordinary bordered pits which connect vertical tracheids (*Figure 7*), the small bordered pits between vertical tracheids and radial ray tracheids, and the large window pits between vertical tracheids and radial ray parenchyma cells (*Figure 8*). The window pits are gaps in the cell wall where the three secondary wall layers S_1, S_2 and S_3 are absent and the window consists of primary wall only. The bordered pits are more complicated. Their structure is best understood from their development. The first sign of the formation of a pit in the young tracheid can be seen in the primary wall at the stage when its surface growth has finished. The network of microfibrils becomes looser at certain places in the primary wall and, at about the same time, new microfibrils are deposited in a circle around the areas of looser network. The microfibrils deposited later in the S_1, S_2 and S_3 layers " flow " around the pits. In the centre of the pit, the structure termed the torus is deposited by secondary thickening of the primary wall. The pits are always formed in pairs on adjacent cell walls. The mechanism which induces the two neighbouring cells to build a pit at the same place and at the same time is not known but there must obviously exist some protoplasmic connection between two adjacent cells. Usually, the bordered pits are functional only for a limited number of years. They are finally blocked by the torus fastening across the opening on one side.

The *chemistry* of the fibre wall has been studied by spectrophotometric as well as by microchemical methods. For the identification of cellulose the electron microscope has also been used. The presence of microfibrils has usually been attributed to the presence of cellulose. It must, however, be pointed out that this conclusion is scarcely permissible as long as it is not known if the hemicelluloses in wood can also form microfibrils.

The distribution of lignin through the fibre wall has been studied most thoroughly. The following techniques have been used: staining reagents, dissolution of the non-lignin compounds, microdissection and chemical analysis, ultra-violet and fluorescence microscopy. All the techniques gave similar results, and it has now been known for some time that between 60 and 90 per cent of the lignin in the wall is localized in the region of the middle lamella and the primary wall. For softwoods, the lignin content lies somewhere near the lower figure and for hardwoods, near the higher

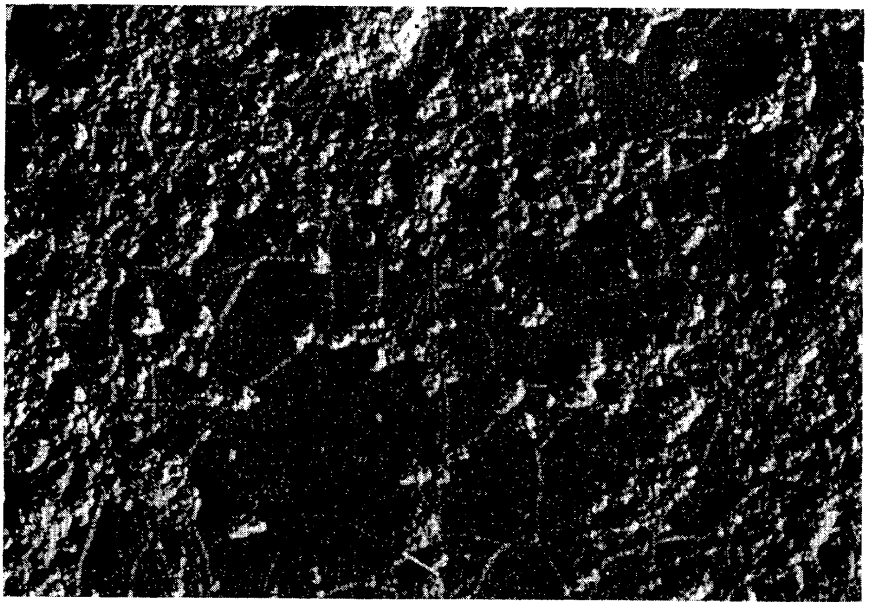

Figure 1. Date endosperm after Turmix and ultrasonic treatment before extraction of mannan *A* (magnification × 45,000)

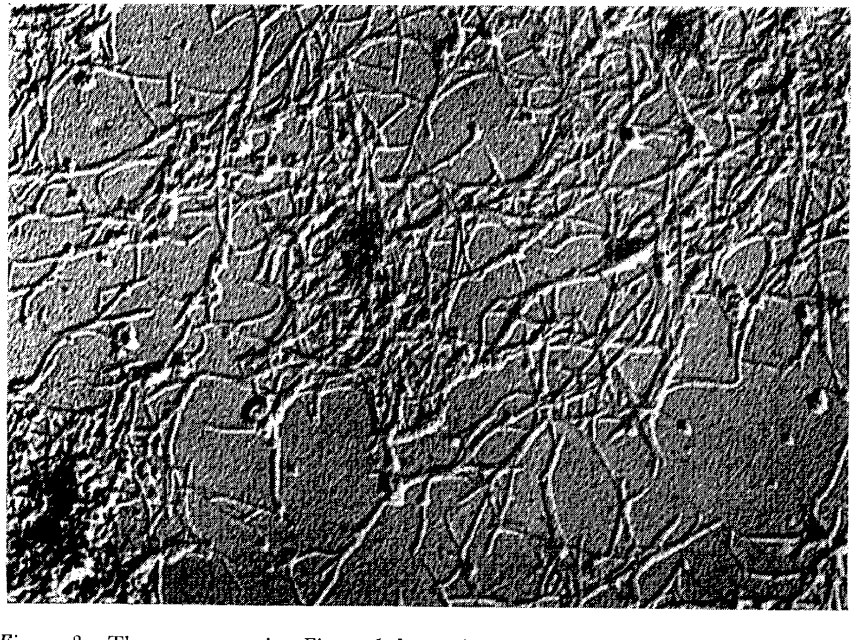

Figure 2. The same as in *Figure 1* but after extraction of mannan *A* (magnification × 37,000)

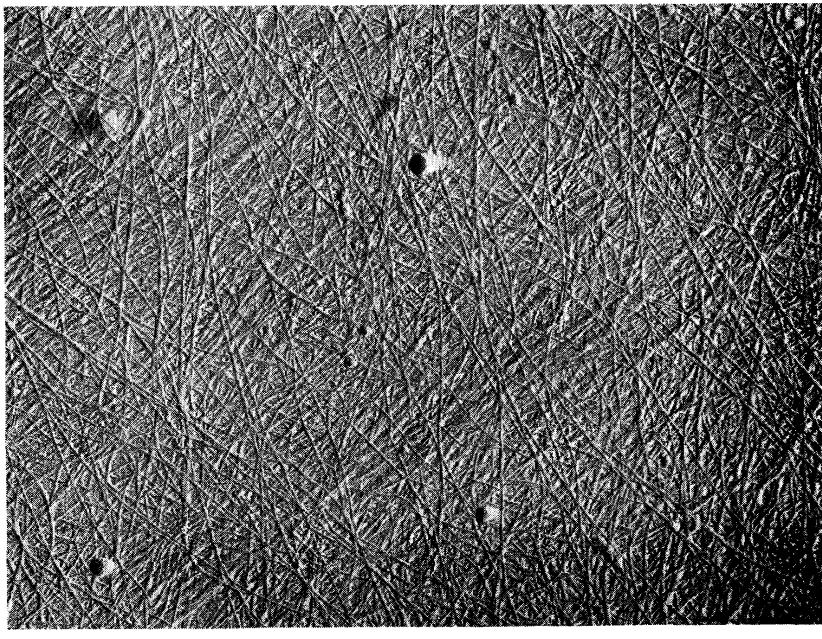

Figure 3. Part of a delignified primary wall (magnification × 25,000)

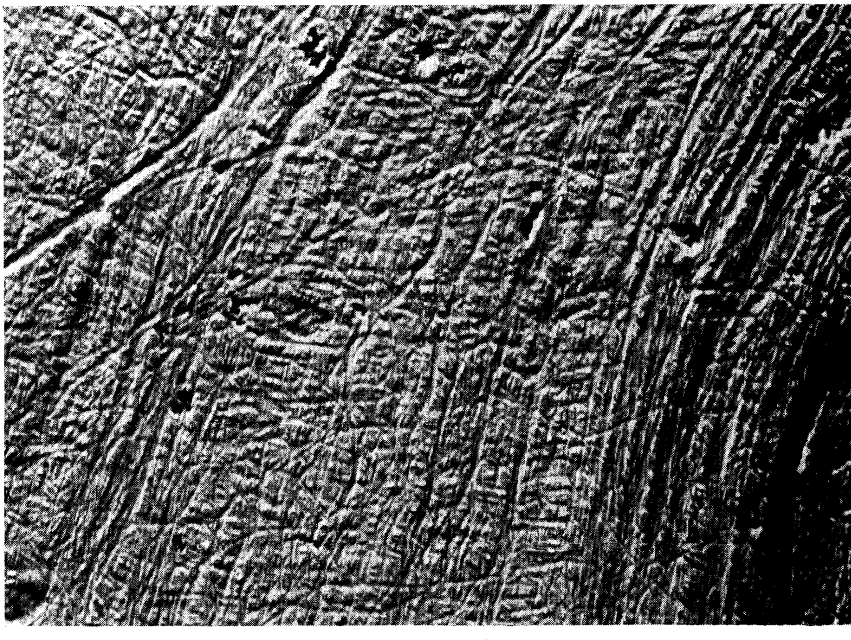

Figure 4. Part of a delignified S_1-layer (magnification × 20,000)

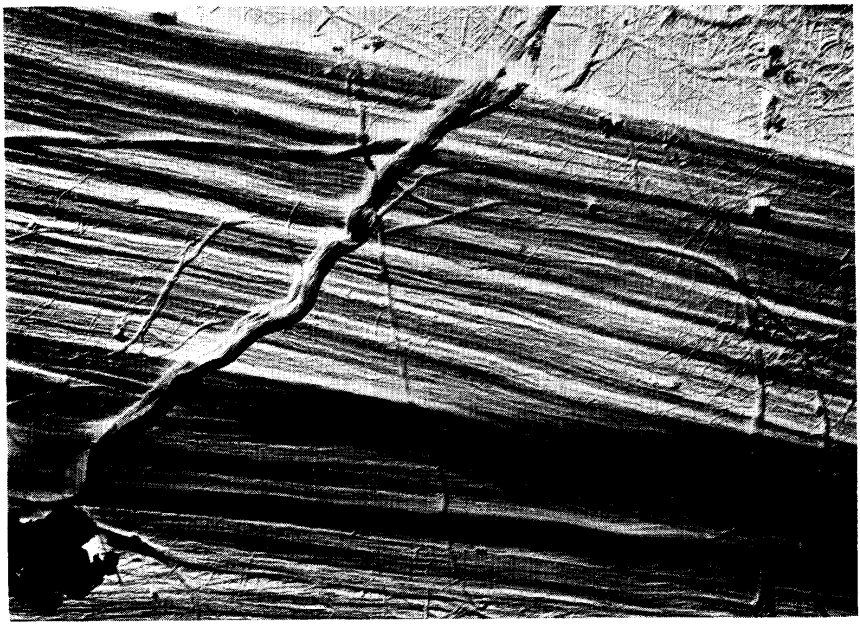

Figure 5. Part of a delignified S_2-layer (magnification × 20,000)

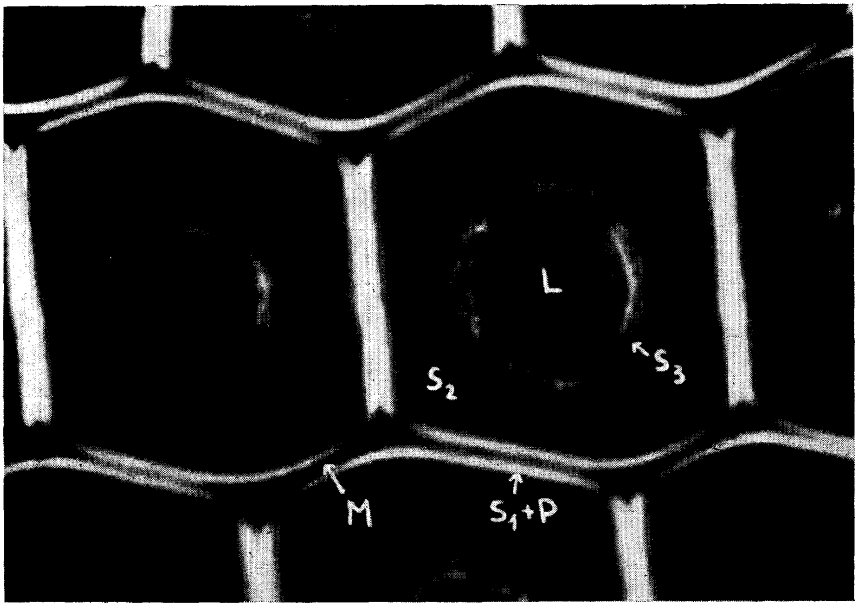

Figure 6. Transverse section through pine wood in polarized light: L = lumen, M = middle lamella, S_1 = outer layer of the secondary wall, S_2 = central layer of the secondary wall, S_3 = tertiary wall (magnification × 3,100)

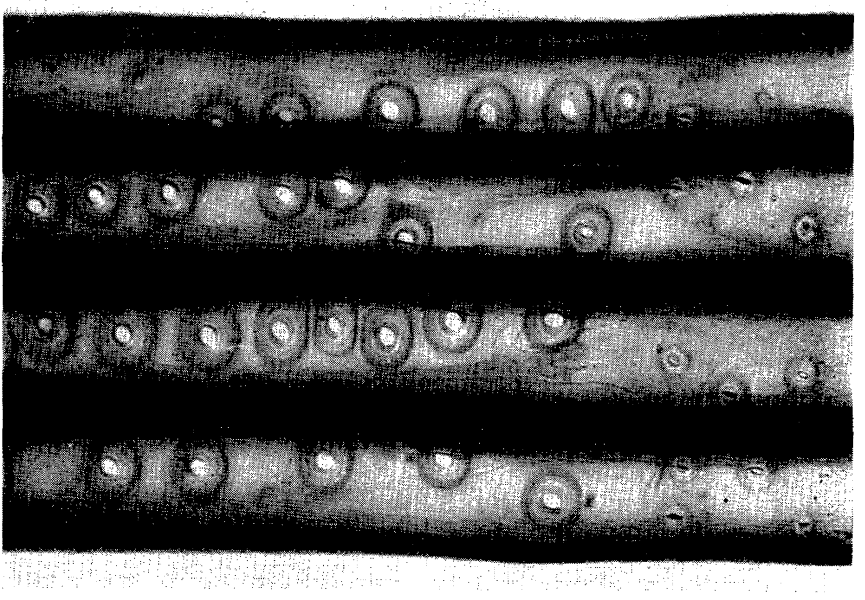

Figure 7. Pine tracheids with bordered pits (magnification × 400)

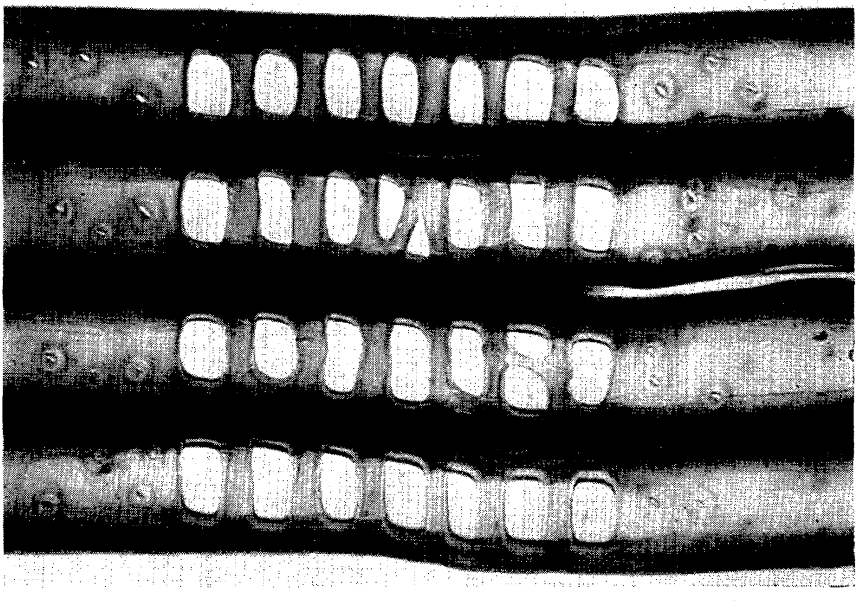

Figure 8. Pine tracheids with large window pits and small bordered pits (magnification × 400)

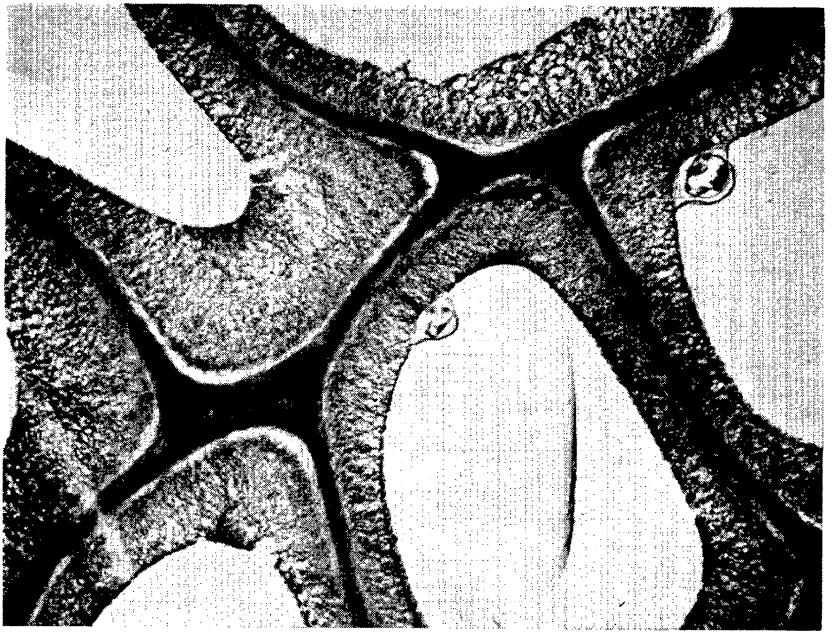

Figure 9. Transverse section through spruce wood; the cellulose has been dissolved to a great extent by a brown rot fungus; the lignin skeleton is left (magnification × 4,500)

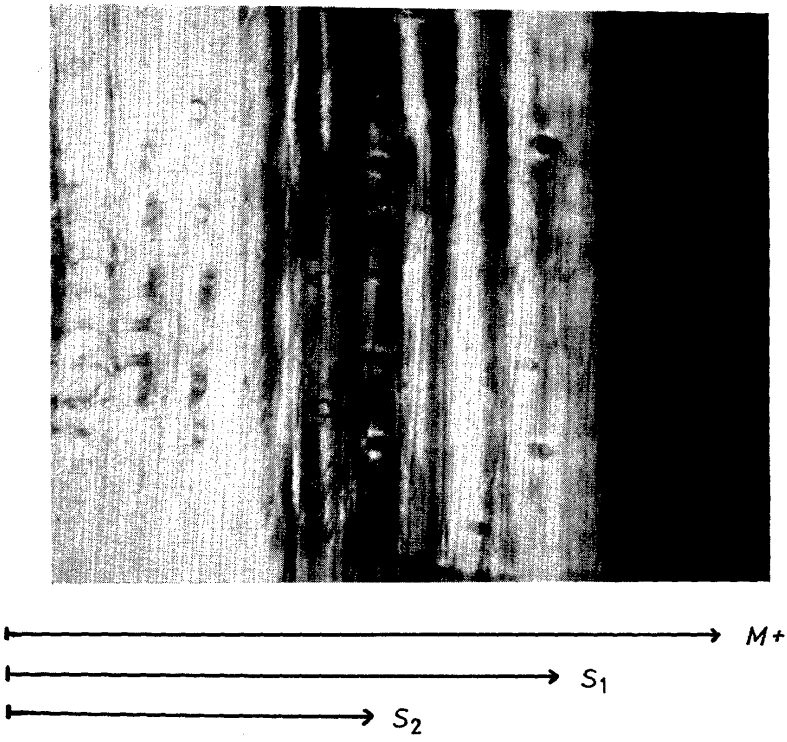

Figure 10. Radial section through the outermost annual ring of the xylem of pine in polarized light; tracheids in different stages of maturation exhibit different optical behaviour (magnification × 380)

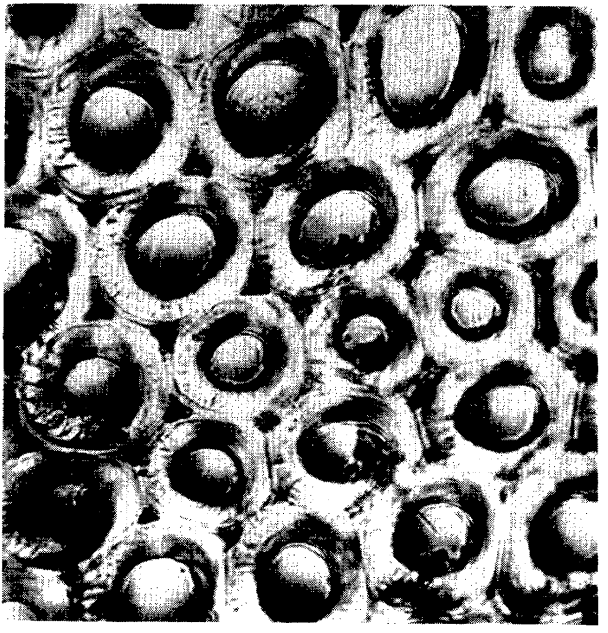

Figure 11. Transverse section through compression wood of pine (magnification × 700)

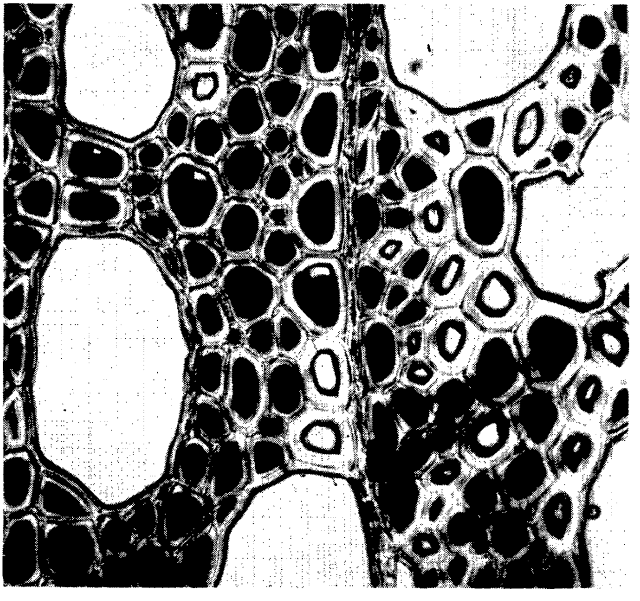

Figure 12. Transverse section through tension wood of aspen; the gelatinous inner layer of the cell walls is swollen and stained with chlor-zinc-iodide (magnification × 350)

one. *Figure 9* gives a rough idea of the lignin distribution in spruce tracheids. The precise location of the border of the heavily lignified middle lamella area and the less lignified secondary wall area is not quite clear. It might lie on the outside, within or on the inside of the S_1-layer.

The location of the *cellulose* and *hemicelluloses* in the fibre wall is more difficult than that of lignin. Kallmes[8] was able, by beating unbleached spruce sulphite fibres, to unravel the $(P + S_1)$-layers and to get two fractions, one being rich in $(P + S_1)$-fragments and the other being rich in $(S_2 + S_3)$-fragments. He found that both fractions differed appreciably from each other in their lignin content but were very similar with respect to their polysaccharide composition. This result, however, does not give much information on the polysaccharide distribution in a *native* fibre. Asunmaa and Lange[9] sought to obtain such information by a microspectrographic method. This, however, involved various drastic pre-treatments of the fibres and the results must, therefore, be interpreted with care. Furthermore, the method suffered from the drawback that it did not enable differentiation between the various types of hemicelluloses. The most reliable technique for studying their location would be the microdissection of single fibres into the single wall layers and the analysis of them after hydrolysis for their sugar composition. Bailey[10], some 25 years ago, isolated the middle lamella of the tracheids of Douglas fir in that way and estimated the lignin microchemically. However, up to now, no-one has succeeded in isolating each of the fibre cell wall layers completely. An alternative approach was, therefore, required and has been devised at the Swedish Forest Products Research Laboratory [11, 12]. During the aging of the young fibre, successive polysaccharide layers are deposited next to one another. Fibres have now been isolated and analysed at various stages of maturation. From a knowledge of the polysaccharides found to be present in these fibre fractions an attempt has been made to deduce the nature of the cell-wall layers in the mature fibre. This approach to the problem entails the assumption that, once a cell-wall layer has been deposited, its polysaccharide composition does not subsequently alter. This is probably true, as the polysaccharides, unlike lignin are deposited on the surface of the protoplast in successive layers.

The young fibres in radial sections through the outermost part of the wood xylem were separated into four fractions: $(M + P)$-fibres, $(M + P + S_1)$-fibres, $(M + P + S_1 + S_2$ outer part) fibres and $(M + P + S_1 + S_2 + S_3)$-fibres. This separation was carried out under the polarizing microscope where the different layers exhibit different optical properties (*Figure 10*). Each fibre fraction was hydrolysed and the sugars were estimated quantitatively. The results were then used to calculate the polysaccharide composition in each of the four fractions.

It is thus possible to deduce very roughly the proportions of the individual polysaccharides in each cell-wall layer. Micrographs of transverse sections taken with the polarizing microscope and with the electron microscope gave a certain volume fraction for each layer in the fibre wall. The density is certainly not the same in all the different layers, but, as a first approximation, the volume percentages can probably be taken as weight percentages. It must, however, be emphasized that this estimate is very tentative and the

Table 1. Percentages of polysaccharides in the different layers of the fibre wall

Polysaccharide	$M + P$*	S_1	S_2 outer part	S_2 inner part + S_3
Birch				
Galactan	16·9	1·2	0·7	0·0
Cellulose	41·4	49·8	48·0	60·0
Glucomannan	3·1	2·8	2·1	5·1
Arabinan	13·4	1·9	1·5	0·0
Glucuronoxylan	25·2	44·1	47·7	35·1
Spruce				
Galactan	16·4	8·0	0·0	0·0
Cellulose	33·4	55·2	64·3	63·6
Glucomannan	7·9	18·1	24·4	23·7
Arabinan	29·3	1·1	0·8	0·0
Glucurono-arabinoxylan	13·0	17·6	10·7	12·7
Pine				
Galactan	20·1	5·2	1·6	3·2
Cellulose	35·5	61·5	66·5	47·5
Glucomannan	7·7	16·9	24·6	27·2
Arabinan	29·4	0·6	0·0	2·4
Glucurono-arabinoxylan	7·3	15·7	7·4	19·4

* Contains also a high percentage of pectic acid.

carbohydrate composition of the different layers as presented in *Table 1* can, therefore, show only the main trends.

For all three woods investigated, the cellulose content is lowest in the $(M + P)$-layer which contains a high percentage of pectic material (galactan, arabinan and pectic acid). There is, however, a marked difference between birch and the two coniferous woods in the arabinan content of the $(M + P)$-layer; in birch it is only half as high as in pine. For the xylan content it is the reverse. In *birch*, the cellulose content is highest in the inner part of the S_2-layer and in the S_3-layer. From the present investigation, however, no conclusion can be drawn about whether S_3 *and* the inner part of S_2 are equally rich in cellulose or whether the cellulose is enriched only in one of them. The S_1-layer and the outer part of the S_2-layer have a very high content of glucuronoxylan.

In spruce and pine the composition of the different cell wall layers is similar. There are some minor differences but these cannot be regarded as characteristic either of spruce or of pine, since they may be due to the use of spring wood tracheids in the case of spruce and of summer wood tracheids in the case of pine. In pine summer wood tracheids the outer part of the S_2-layer is richest in cellulose. The glucomannan content increases steadily from the outer parts of the cell walls to the inner parts. The glucurono-arabinoxylan content is very high in the S_3-layer. The same was found by Bucher[13], who made a controlled hydrolysis of the tertiary wall in spruce wood. There seems to be practically no arabinan in the secondary wall layers of spruce and pine. The galactan value in these layers may be due to the presence of a galacto-glucomannan.

The uneven distribution of the chemical compounds through the single fibre wall also reflects on the chemical differences in distinct anatomical elements in wood. This is clearly shown in spring wood and summer wood. Spring wood fibres and summer wood fibres differ mainly from each other by the much thicker S_2-layer in the latter. The lignin-rich middle lamella makes up a larger portion of the spring wood than of the summer wood, which, therefore, has a somewhat lower lignin content than the former. Another consequence, as is shown in *Table 2*, is that summer wood has a higher glucomannan and a lower glucuronarabinoxylan content than spring wood, where the glucomannan-rich S_2-layer makes up a lower percentage and the glucuronarabinoxylan-rich S_3-layer a higher percentage than in summer wood.

Table 2. Relative percentages of polysaccharides in spring wood and summer wood from pine (*P. Silvestris L.*)

Polysaccharide	*Spring wood*	*Summer wood*
Galactan	3·4	3·1
Cellulose	56·7	56·2
Glucomannan	20·3	24·8
Arabinan	1·0	1·8
Glucurono-arabinoxylan	18·6	14·1

Besides the vertical tracheids and fibres, the ray cells are an important anatomical entity in both softwoods and hardwoods. Their volume percentage is a few per cent in softwoods rising to some 30 per cent in certain hardwoods. Perilä and co-workers[14] have studied the chemical composition of ray cells from spruce, pine and birch. In spruce and pine they found that the ray cells contained about the same amount of total hemicellulose as the vertical tracheids, but the total hydrolysate of the tracheids was found to be richer in mannose and poorer in xylose than the hydrolysate of the ray cells. The ray cells from birch had an entirely different composition from the fibres and the vessels. Whereas the glucuronoxylan content in the latter is about 2/3 of the cellulose content, the ray cells contain almost four times as much glucuronoxylan as cellulose.

Most stems, hardwood as well as softwood, contain a smaller or larger amount of reaction wood, *i.e.* wood which has grown under tension or compression. In leaning stems or in stems which grow on places which are very much exposed to wind, the percentage of reaction wood can be rather high. It has been known for some time that reaction wood fibres differ morphologically as well as chemically from normal wood fibres. *Figure 11* shows a transverse section through compression wood from pine. This is characterized by rounded tracheids with well-developed intercellular spaces and with fissures in the cell wall. The lignin content in compression wood from pine is as high as 40 per cent. The carbohydrate composition is shown in *Table 3*. The very high galactose content which was found to originate from a β-1,4-linked galactan with some galacturonic acid residues linked to it[15] is most characteristic.

Table 3. The carbohydrate composition of normal wood and of compression wood from spruce (*Picea Abies Karst.*)

	Galactose	*Glucose*	*Mannose*	*Arabinose*	*Xylose*
Normal wood	2·1	70·7	17·2	1·3	8·7
Compression wood	19·6	55·6	11·5	2·6	10·7

The tension wood fibres in hardwoods are characterized by a gelatinous cell wall layer bordering the lumen (*Figure 12*). This layer seems to consist almost exclusively of cellulose and is obviously responsible for the higher cellulose, the lower lignin and the lower glucuronoxylan content of tension wood, as is seen in *Table 4.*

Table 4. The carbohydrate composition of normal wood and of tension wood from beech (*Fagus Silvatica L.*)

	Galactose	*Glucose*	*Mannose*	*Arabinose*	*Xylose*
Normal wood	1·6	57·4	4·9	1·0	35·1
Tension wood	6·6	73·5	—	2·6	17·3

Our knowledge of the physical and chemical fine structure of wood has greatly increased in recent times. The biological processes which produce these structures are, however, still rather obscure, and it appears that it will need considerable efforts to throw some light on this side of the subject. But we may hope that the research, which up to now has been directed very much to the investigation of the mature, dead wood fibres, will, in the future, be directed more and more to the cambial region of the stem, *i.e.* to that region where things happen.

References

[1] H. Ambronn and A. Frey. *Das Polarisationsmikroskop* Akademische Verlagsgesellschaft Leipzig (1926).
[2] K. Mühlethaler. *Beih. Z. Schweiz. Forstverein* **1960**, No. 30, p. 55.
[3] H. Meier. *Biochim. et Biophys. Acta* **28**, 229 (1958).
[4] C. Y. Liang, K. H. Bassett, E. A. McGinnes and R. H. Marchessault. *Tappi* **43**, 1017 (1960).
[5] J. Cronshaw, A. Myers and R. D. Preston. *Biochim. et Biophys. Acta* **27**, 89 (1958).
[6] H. Bucher. *Holzforschung* **11**, 97 (1957).
[7] W. Liese. *Holz Roh- u. Werkstoff* **18**, 296 (1960).
[8] O. Kallmes. *Tappi* **43**, 143 (1960).
[9] S. Asunmaa and P. W. Lange. *Svensk Papperstidn.* **57**, 501 (1954).
[10] A. J. Bailey. *Ind. Eng. Chem., Anal. Ed.* **8**, 52 (1936).
[11] H. Meier and K. C. B. Wilkie. *Holzforschung* **13**, 177 (1959).
[12] H. Meier. *J. Polymer Sci.*, **51**, 11 (1961).
[13] H. Bucher. *Das Papier* **14**, 542 (1960).
[14] O. Perilä. *J. Polymer Sci* **51**, 19 (1961).
[15] H. O. Bouveng and H. Meier. *Acta Chem. Scand.* **13**, 1884 (1959).