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# The Cellular Distribution of Lignans in *Tsuga heterophylla* Wood*

By R. L. KRAHMER, R. W. HEMINGWAY and W. E. HILLIS** C. S. I. R. O.

Forest Products Laboratory, Division of Forest Products, C. S. I. R. O., South Melbourne

## Summary

Western hemlock heartwood contains patches of tracheids with large amounts of cellular inclusions. Microscopic and chemical examination of the wood showed several types of deposits containing the lignans matairesinol, hydroxymatairesinol and conidendrin. The deposits, which were often relatively pure individual lignans, frequently assumed different physical forms and chemical composition. A check in the wood contained three distinct forms of deposits each of which was a different lignan. Rays contained deposits physically similar to those in adjacent tracheids but, while lignans were present in the tracheids, they were not detected in the rays. Lignans lined tracheid walls as surface films and often encrusted the bordered pits. The amount of lignans in the wood was not related to wet wood zones although surface films and pit encrustations should have an influence on physical properties. The location and physical nature of lignan deposits in western hemlock heartwood indicates their biosynthesis has probably taken place at the heartwood periphery in the vicinity of the half-bordered pit.

## Introduction

Heartwood, and even sapwood in certain instances, contains many extractable materials that have been deposited in particular locations. Studies have been made on the possible relationship between certain wood properties (e.g. permeability to fluids) and the anatomical or morphological characteristics of particular deposits observed with light and electron microscopes. There have been few examinations of the particular position of specific compounds in wood or of their purity or location in wood cells.

Western hemlock (*Tsuga heterophylla* (Raf.) Sarg) extractives have been studied in detail by BARTON and coworkers [1961, 1966, 1969] as well as GOLDSCHMID and HERGERT [1961]. The lignans matairesinol (I), hydroxymatairesinol (II), and conidendrin (III) were normal components of heartwood and only small amounts were found in sapwood [BARTON, GARDNER 1966]. The largest concentration of hydroxymatairesinol occurred near the sapwood-heartwood boundary and it was found in only negligible amounts in the sapwood. GOLDSCHMID and HERGERT [1961] have shown that hydroxymatairesinol is present in hemlock sapwood at fives times the concentration of conidendrin; BARTON [1963] found the same ratio in heartwood.

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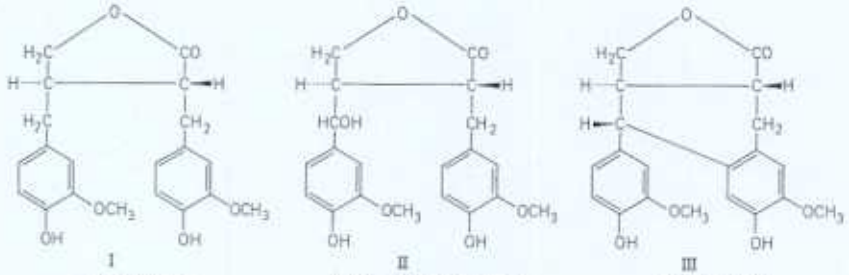


I Matairesinol

II Hydroxymatairesinol

III Conidendrin

White flecks visible to the naked eye can be seen frequently in the wood of western hemlock and these have been called floccosoids [GERRY 1943; GRONDAL, MOTTET 1942]. They are unsymmetrical in shape, common but randomly scattered in heartwood and have not been reported in sapwood. Floccosoids resemble the white decay pockets caused by *Fomes pini* (Thore) Lloyd but GERRY [1943] has indicated that the deposits responsible for the flecks are extractable and that enzymatic degradation of the wood cells is not evident. Microscopic examination of floccosoids [BARTON 1963] has shown that these deposits largely fill the lumens of tracheids but they were not evident in the ray cells. BARTON [1963] has shown that these deposits were almost completely α-conidendrin with neither matairesinol nor hydroxymatairesinol being detectable by paper chromatographic methods. In addition to the occlusions described as floccosoids, whitish deposits have also been reported in ring shakes in mountain hemlock (*Tsuga mertensiana* (Bong.) Carr.), *Abies amabilis* (Dougl.) Forbes and western hemlock [BARTON, GARDNER 1961, 1966; MEYER, LENEY 1968]. These deposits have been identified as matairesinol in all these species. Also, BARTON [1966] has found the crystals in water shakes of *T. heterophylla* to be largely matairesinol with small amounts of hydroxymatairesinol.

Western hemlock heartwood also contains wet pockets (150% moisture content compared with 65% for normal heartwood) which cause serious difficulties during drying. In a study concerned with the drying rate of wet pockets in western hemlock, KOZLIK and SCHROEDER [1968] found that acetone extraction raised the drying rate of wet wood. They reported a considerably higher amount of acetone soluble material in wet wood zones, and the extracted material was mainly conidendrin.

A study of the cellular distribution of lignans in western hemlock heartwood should be of value for interpreting aspects of heartwood biochemistry and for determining possible effects of these heartwood constituents on wood properties. This paper reports our study of the lignans matairesinol, hydroxymatairesinol and conidendrin in woody tissues.

## Materials and Methods

### Sample Material

Samples of western hemlock wood having wet pockets and observable floccosoids were collected by the School of Forestry, Oregon State University, U.S.A., and sent by air to the Division of Forest Products, C.S.I.R.O., Melbourne,

Australia. Most samples were radial strips extending from pith to cambium. The material was taken from recently felled trees, wrapped in plastic and stored in a cold room (0 ... 4° C) during study.

## Methods

The techniques used for analyses involved light and electron microscopy, thin-layer (TLC) and paper chromatography (PC), gas-liquid chromatography (GLC), and ultraviolet (UV) absorption, including use of a microspectrophotometer (MSP).

### *Microscopy*

The deposits in the cells and tissues of western hemlock were examined with both light and electron microscopes. For the study of the general morphology of deposits in the fine structure of wood cells, a Siemens II electron microscope was used. Most observations were made on direct replicas, prepared according to the technique described by CÔTÉ, KORAN and DAY [1964]. Photomicrographs of most deposits were taken with a Zeiss Ultraphot II light microscope. For low magnifications, a Wild stereoscope (magnification range 60 ... 200 X) was used to observe split and microtomed surfaces of wood. This microscope was also used to observe the removal of minute deposits from cells, and the dissection of particular cells and tissues from the wood for chemical analyses. Most samples of deposits for GLC studies were obtained from air-dried microtome sections approximately 20 μm thick. Dissection of small pieces of the section containing deposits was done with stainless surgical blades (e.g. Swann-Morton, England), whereas removal of minute deposits from sections was accomplished with a tungsten wire probe on which a fine point had been made by electrolysis [BRADY 1965]. The small samples were placed in a 2 cm long capillary tube having an inside diameter of about 0.8 mm.

### *Chromatography*

The small deposits of lignans in the wood cells were analysed by GLC. A Varian 2100 gas chromatograph with flame ionization detectors was used. Columns were 2 metres long of 3 mm inside diameter glass U tubes packed with 3.6% Apiezon L on 80 ... 100 mesh DMCS Chromosorb W. The oven temperature was 230° C, and detector and injection temperatures were 255 °C. The carrier gas (helium) flow rate was 40 ml/min, while hydrogen and air flow rates were 25 ml/min and 250 ml/min. Trimethylsilyl (TMS) ethers were prepared by reacting samples 5 ... 10 minutes at room temperature with hexamethyldisilazane and trimethylchlorosilane in pyridine (2 : 1 : 10 parts by volume). Calibration curves for the three lignans indicated linear response from at least $25 \times 10^{-7}$ to $1 \times 10^{-8}$ grams of lignan injected.

Relative retention times obtained from the above chromatographic conditions were: — TMS-matairesinol 1.00; TMS-hydroxymatairesinol 0.76; TMS-conidendrin 1.25. Because pinoresinol and oxomatairesinol were also found in western hemlock sapwood [GOLDSCHMID, HERGERT 1961] their relative retention times were determined and found to be 2.10 and 1.23, respectively. The retention time for TMS-oxomatairesinol is nearly the same as that for TMS-conidendrin. Oxomatairesinol is an auto-oxidation product of hydroxymatairesinol [GOLDSCHMID,

HERGERT 1961] and its amounts in fresh wood should be of minor proportions. No spot corresponding to oxomatairesinol was found on TLC plates. GLC examination of silylated acetone extracts from which conidendrin had been removed (by preparative TLC) indicated little or no formation of oxomatairesinol. Unidentified substances obtained from TLC plates did not interfere with GLC peaks of the relevant lignans. Many different GLC liquid phases were examined but none gave as good resolution of the lignans as Apiezon L.

The silylating reagent (3 ... 10 µl) was added to the sample in the capillary tube, allowed to stand 5 ... 10 minutes at room temperature then injected directly into the gas chromatograph without separation of the ammonium chloride precipitate. Comparison of GLC peak areas with the calibration curves indicated that many deposits analysed weighed approximately $5 \times 10^{-9}$ grams. Other samples were prepared by placing portions of microtome sections in the capillary tubes and reacting the wood sections with silylating reagent. Addition of wood to the reaction mixture did not interfere with the qualitative aspects of the analysis.

TLC plates of Kieselgel GF 254 (E. Merck AG, Darmstadt) were prepared with thickness of 0.25 mm for analytical work and 0.75 mm for preparative work. The solvents used together with $R_f$ values for the lignans are shown in Table 1.

Table 1. *$R_f$ Values of Lignans by TLC*

| Solvent | Lignan* | | | | |
|---|---|---|---|---|---|
| | Conid. | Mat. | OH Mat. | Oxomat. | P'resinol |
| Chloroform: acetic acid (4 : 1) | 0.87 | 0.87 | 0.55 | 0.82 | 0.92 |
| Chloroform: ethyl acetate (7 : 4) | 0.54 | 0.52 | 0.25 | 0.30 | 0.46 |
| Toluene: ethyl acetate: formic acid (5 : 4 : 1) | 0.63 | 0.62 | 0.56 | 0.60 | 0.62 |
| Benzene: ethanol (150 : 22) | 0.45 | 0.48 | 0.40 | 0.45 | 0.48 |
| Benzene: acetone (3 : 2) | 0.71 | 0.72 | 0.54 | 0.65 | 0.70 |

* Conid. = conidendrin; Mat = matairesinol; OH Mat = hydroxymatairesinol; Oxomat. oxomatairesinol; P'resinol = pinoresinol.

Spots were detected under short wave UV light or by spraying with either a saturated solution of diazotized p-nitroaniline in 20% sodium acetate or diazotized sulphanilic acid followed by 20% sodium carbonate. None of the solvents separated matairesinol from conidendrin. The presence of matairesinol was evaluated by paper chromatography. The matairesinol-conidendrin zone was scraped from the TLC plates, washed with acetone and the filtrate evaporated under vacuum. The filtrate was then spotted on Whatman no. 1 papers and chromatographed in the ascending direction with either 6% acetic acid in one dimension or n-butanol: acetic acid: water (6 : 1 : 2; "BAW") followed by 6% acetic acid in the second dimension.The $R_f$ values of conidendrin and matairesinol in 6% acetic acid were 0.00 and 0.65 respectively and in BAW were 0.79 and 0.86. Spots on paper chromatograms were detected with diazotized sulphanilic acid followed by sodium carbonate.

*Spectroscopy*

The IR spectra were kindly obtained by A. J. MICHELL on a Grubb Parsons double beam spectrophotometer using KBr discs in a Perkin Elmer Microsampling Unit. The UV spectra of methanol solutions of lignans before and after addition

of 0.5 N NaOH were obtained using a Unicam SP-800 spectrophotometer. Identification of deposits as lignans in particular wood cells was made by analyzing the UV absorption of deposits in situ with a modified Olympus A-4 microspectrophotometer. Thin wood sections were cut with a sliding microtome and mounted in glycerin on quartz slides. A 2 ... 3 μm spot of light from a deuterium lamp was oriented to pass through particular deposits, and a wave-length scan was made from 250 ... 310 nm, the absorbance being automatically recorded on a chart. Because the energy level of the UV beam increased during this wave-length scan, a series of resistors was incorporated into the system so that a base line of constant transmittance could be set for a quartz slide in which only glycerin was the material in the UV path. For study of deposits located in cell lumens, wood sections 6 ... 8 μm thick could be used because the deposits were of variable thickness, and adequate transmittance of the UV beam was possible through much of the deposited material. Similar transmittance through the cell wall required transverse sections to be about 2 μm thick.

## Results

Microscopic observation of western hemlock heartwood revealed several different physical forms of deposits. Tracheids filled with a white crystalline deposit, previously described as floccosoids, were readily observed. In addition, large deposits of a colourless, clear material were also observed to block tracheid lumens although they occurred less frequently than the floccosoids. Ray cells contained deposits physically similar to floccosoids along with more frequently occurring

Table 2. *Composition of Various Deposits in Western Hemlock*

| Type of Deposit | Compound | | | | |
|---|---|---|---|---|---|
| | Mat.[1] % | OH Mat.[1] % | Conid.[1] % | Unknown "0.53" % | Unknown "0.65" % |
| Floccosoid | 0 ... 1 | 0 ... 10 | 90 | 0 ... 4 | 0 ... 5 (some large)[2] |
| Clear | 0 ... 1 | 80 | 5 | 2 ... 15 | trace |
| Other White Deposits | | | | | |
| a | trace | 20 ... 30 | trace | 60 ... 80 | n. d.[3] |
| b | trace | 80 ... 90 | n. d. | trace | n. d. |
| Cell Wall Lining | | | | | |
| a | small | 50 | 50 | n. d. | some large |
| b | n. d. | n. d. | n. d. | large | n. d. |
| Within Cell Wall | n. d. | small | n. d. | small | n. d. |
| Rays | n. d. | n. d. | n. d. | small | n. d. |
| Check | | | | | |
| Mixture | 85 ... 90 | 2 | 2 | 5 | n. d. |
| Chunky form | 95 | n. d. | 5 | n. d. | n. d. |
| Spherical form | trace | n. d. | 80 | 10 | n. d. |
| Feathery form | 3 | 95 | n. d. | n. d. | n. d. |

* Relative composition based on proportions of peak areas in gas liquid chromatograms. Representative values are expressed as variation between samples was sometimes large.

[1] See footnote Table 1.

[2] Acetone extracts of some wood samples contained larger amounts of this compound.

[3] Not detected.

yellow globules. Observation at higher magnification showed that tracheids were often lined with coatings of acetone soluble materials and bordered pits were sometimes completely blocked in these areas. A check in a heartwood sample was filled with deposits of several shapes. A white chunky material predominated along with some spherical and feathery white deposits. Analyses of these deposits are detailed below and a summary of chemical findings is shown in Table 2.

## Examination of Tracheids

### *Floccosoids*

The white flecks that were readily seen on the surface of hemlock wood (Fig. 1) were due to longitudinal tracheids that were largely occluded with white deposits. These tracheids were usually in clusters, but they did not appear to form a definite shape on the transverse surface, although the distance across the floccosoid deposit did not generally exceed 2 ... 3 mm. Also, most floccosoids were less than one cm along the grain. Examples of photomicrographs of both transverse and radial longitudinal sections containing these floccosoids are given in Fig. 2. It will be noticed that the boundaries of the floccosoids are abruptly defined. Examination of these deposits in situ with the MSP yielded a guaiacyl lignan UV absorption spectrum (Fig. 3) and this was also obtained from dissected deposits using a Unicam SP 800 spectrophotometer. The curves obtained with the MSP are compressed in comparison with that from the Unicam SP 800 because of lack of power in the peak absorption regions. The infrared spectra of deposits dissected from wood or analysed in situ showed remarkable similarity to that of pure conidendrin. Chromatographic examination (TLC and PC) of acetone extracts of wood contain-

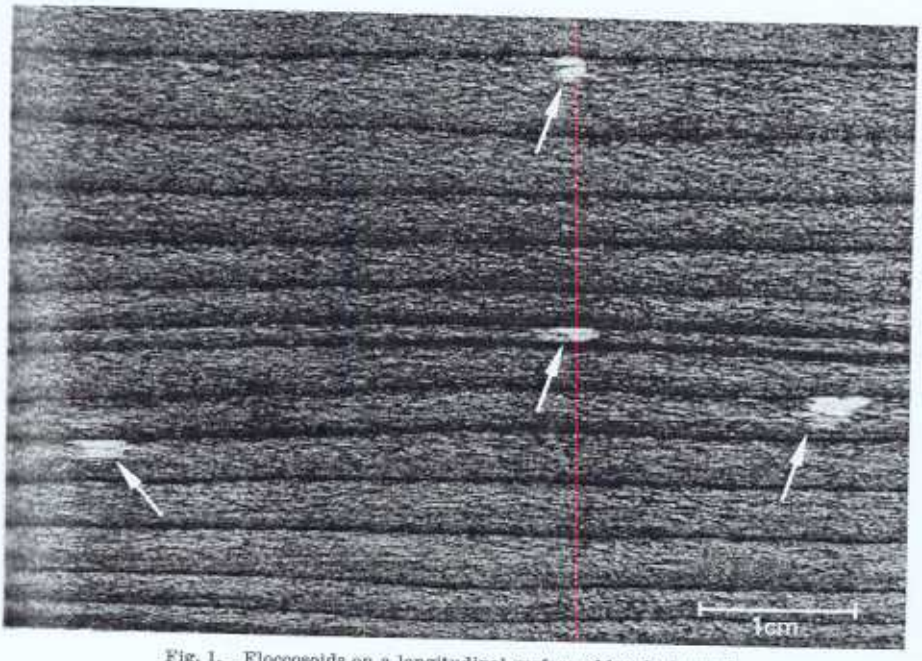



Fig. 1. Floccosoids on a longitudinal surface of hemlock wood

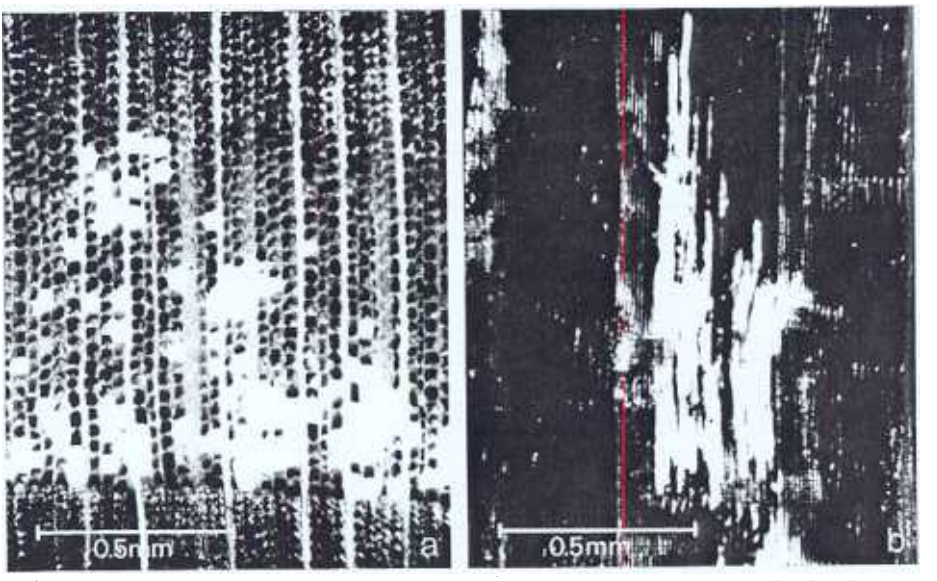
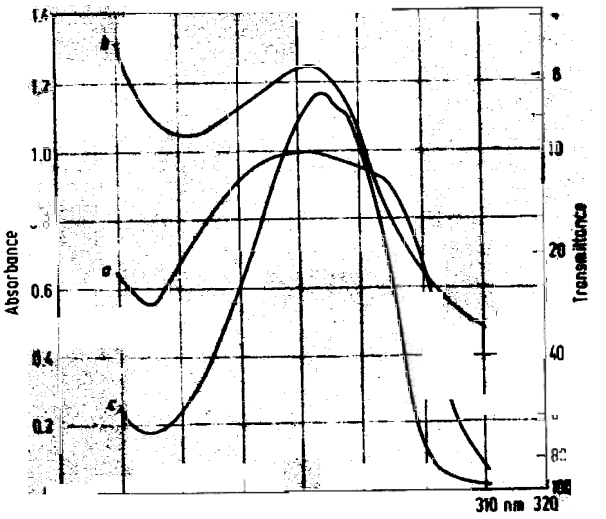



Fig. 2. Microscopic appearance of floccosoids on transverse (a) and radial longitudinal surfaces




ing floccosoids also indicated that these deposits were largely conidendrin. GLC examination of several micro samples (about $10^{-8}$ gram) from individual tracheids showed a high degree of purity of conidendrin (Fig. 4). Floccosoids dissected from tracheids at varying distances from the heartwood boundary to the pith were notably similar in composition. All samples contained about 10% of an unknown substance with relative retention time 0.53. (This compound will be referred here

after as unknown 0.53 and the estimates of amounts are based solely on relative peak areas). When a large sample was analysed by GLC at high sensitivity, some (up to 10%) hydroxymatairesinol and matairesinol (less than 1%) were detected in the occlusions. The analysis of several samples showed that the general composition of the floccosoid deposits was the same whether the occlusion was removed from the wood and analysed or whether a number of tracheids containing the deposits were treated directly for GLC analysis. These results support the view that hydroxymatairesinol and matairesinol occur mainly in the conidendrin occlusions and not solely as a lining of the tracheid walls.

Some samples contained small but significant amounts of an unknown substance with a relative retention time of 0.65. This compound was common in samples of acetone extracts of the wood. An unknown compound obtained from preparative TLC plates had a similar relative retention time on GLC (0.65). This compound had a UV absorption spectra of the guaiacyl type of lignan, it became brown with diazotized *p*-nitroaniline and pink with diazotized sulphanilic acid. TLC showed it was not oxomatairesinol or pinoresinol; with chloroform-acetic acid it had $R_f = 0.69$, PC in 6% acetic acid gave an $R_f$ value of 0.58 while matairesinol had $R_f = 0.65$.

### *Clear Deposits*

In contrasting appearance to the white opaque floccosoids there were also clear colourless deposits in clusters of tracheids. These tracheids were observed near the sapwood-heartwood boundary in two specimens from different trees. Sometimes the deposits were large enough to block the lumen (Fig. 4) but this situation occurred less frequently than with floccosoids. UV absorption spectra of the clear deposits obtained with the MSP were typical lignan spectra (Fig. 3). In regions containing only the clear deposits GLC showed that hydroxymatairesinol was the main component with small amounts of conidendrin and unknown 0.53 (Fig. 4). Larger samples showed that conidendrin was about 4% and matairesinol about 1% of hydroxymatairesinol. Unknown compound 0.53 was also present in most samples from this zone at from 2 ... 15% of the hydroxymatairesinol. Frequently with these deposits a white coating could be seen on the cell walls and in these cases the amount of conidendrin was increased although the major compound present was still hydroxymatairesinol.

### *White Deposits*

Close to the zones containing the clear deposit some white materials were also observed in tracheid lumens. They differed from floccosoid deposits in that they usually contained 2 ... 4 times as much of the unknown compound 0.53 as hydroxymatairesinol which in turn was much greater than the amounts of matairesinol and conidendrin present. Other whitish deposits were largely hydroxymatairesinol so this compound is not exclusively associated with the clear deposits.

### *Cell Wall Linings*

The cell walls of many tracheids in areas free from floccosoids were lined with a thin layer of material. It was noticeable both in light and electron microscopy, and the micrographs (Fig. 5) illustrate the thin coating partially or fully covering

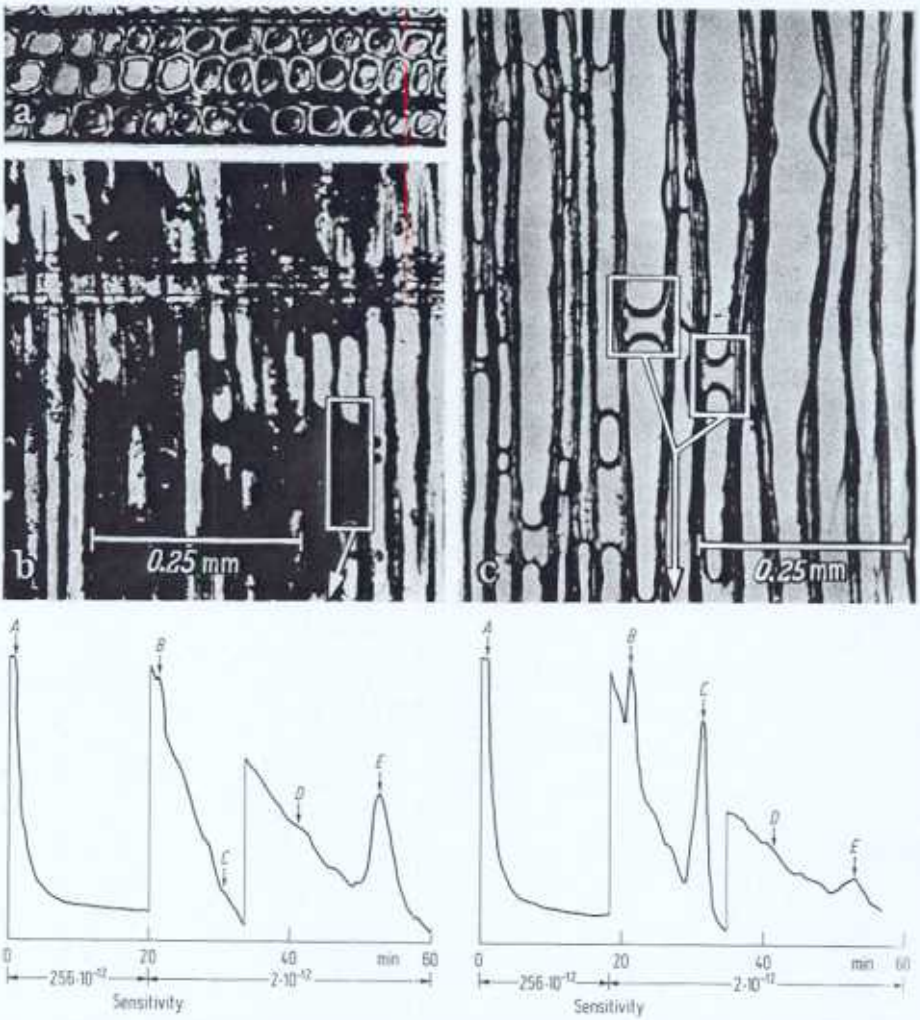



Fig. 4. Microanalysis of deposits in the heartwood of western hemlock by highly sensitive gas liquid chromatography.

The minute deposits marked in photomicrographs (b) and (c) were removed from these radial longitudinal sections and identified. The chromatograms below each photomicrograph show the position and magnitude of peaks representing compounds in the circled deposits. The heavily occluded tracheids shown in transverse section (a) and longitudinal section (b) contain mainly conidendrin (E), whereas the transparent deposits in section (c) consist of hydroxymatairesinol (C) and smaller quantities of an unknown compound (B) and conidendrin (E). Chromatographic positions of matairesinol (D) and the solvent (A) are also shown

the warty layer and showing the type of blockage that can occur in and around the bordered pits. Acetone extraction of matched samples removed these deposits (Fig. 5).

A comparison was made of the composition of a portion of tracheids filled with floccosoids with portion of the adjacent tracheids lacking these deposits. The occluded tracheids contained largely conidendrin and a small amount of hydroxymatairesinol in an approximate ratio of 130 : 1. The "empty" tracheids contained

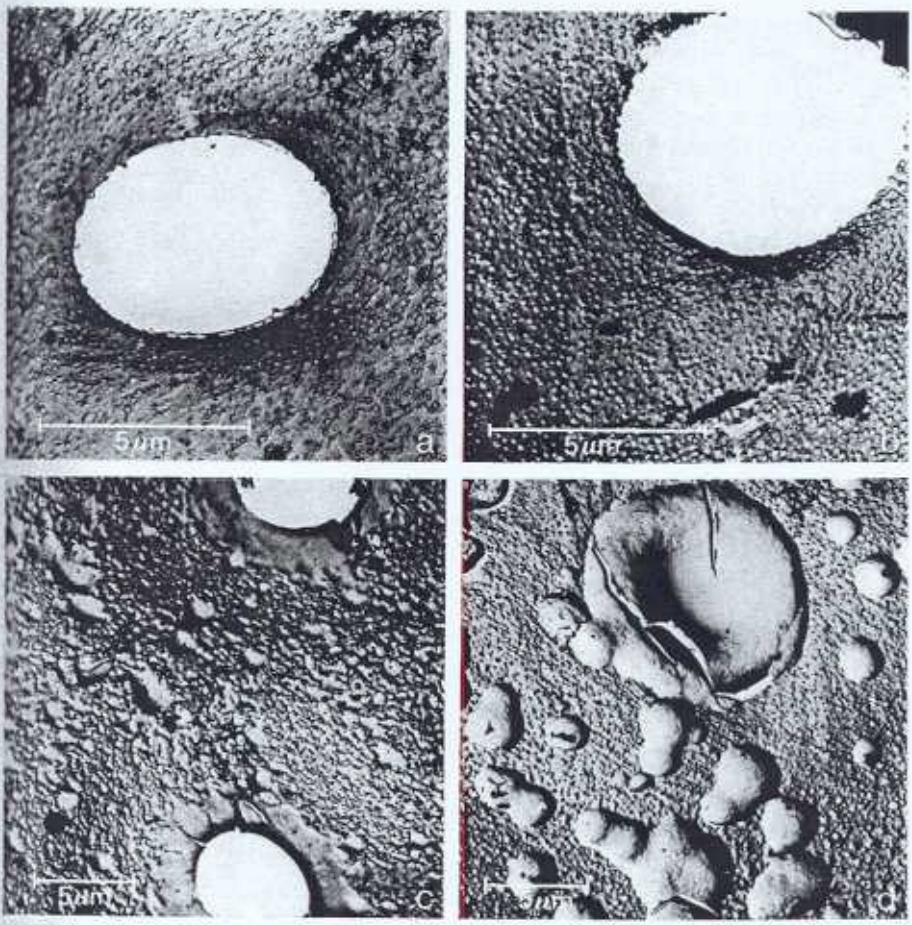



Fig. 5. Deposits observed on tracheid walls in western hemlock. A thin film covering the warty layer on the border of a pit pair (a) was removed by acetone extraction (b). Larger deposits are observed on tracheid walls and in and around the pit apertures in (c) and (d)

much smaller amounts of lignans but their ratio was different and approximately of the order of 1 : 1.

Analysis of cell wall surfaces adjacent to the lumen with the MSP usually gave mixed lignan and lignin absorption curves even in areas remote from the large deposits described above. Matairesinol, hydroxymatairesinol and conidendrin were all found by GLC in tracheids in which deposits were not observed by optical microscopy. In one of these samples considerable amounts of the unknown 0.53 were found but no peaks for the three lignans were observed. The data obtained from electron microscopy, MSP and GLC suggest that most if not all heartwood tracheid walls are lined with lignans to a varying extent whereas the large deposits, such as floccosoids, occur only irregularly.

### *Lignans in the Cell Wall*

The absorption spectra of 2 μm areas incorporating the middle lamella were determined by the MSP (Fig. 3). The spectra of the secondary walls differed from

those of the middle lamella in that there was an increased absorption in the longer wave length regions and particularly at 290 nm which is a characteristic of lignan curves (Fig. 3). This absorption in the secondary wall due to lignans remained even after acetone extraction.

Gas liquid chromatography of silylated wood sections after various extraction procedures showed that the lignans were difficult to remove. Cold benzene extraction of microtome sections removed very little if any of the lignans and even after hot benzene extraction there remained small amounts of the three lignans and the unknown 0.53. Although the lignans are very soluble in cold acetone, small amounts of hydroxymatairesinol and the unknown 0.53 remained after microtome sections had been boiled in four exchanges of acetone.

## Examination of Rays

The rays contained small amounts of yellow globules and chunky deposits physically similar to floccosoids (Fig. 6). The comparison of micro samples of rays containing the ray-tracheid crossing walls with samples of the adjacent tracheids containing floccosoids indicated that the proportions of conidendrin, matairesinol and hydroxymatairesinol were the same. Thus, the rays either contained no lignans and the lignans obtained in the analysis were from the tracheid side of the ray crossing or the lignans in the rays were present in the same proportion as in the adjacent tracheids.

Walls of ray cells that did not include the ray crossings with longitudinal tracheids were dissected from radial sections of 6 μm thickness. GLC examination revealed the presence of the unknown 0.53, several unknown compounds with relatively low retention times, but no hydroxymatairesinol, matairesinol or conidendrin. GLC analysis of the tracheids just below these ray cells showed the presence of a large amount of hydroxymatairesinol and less conidendrin along with the unknown 0.53. The deposits in many ray cells adjacent to occluded tracheids were examined by the MSP but none gave lignan spectra. In one section the ray cell and its adjacent tracheid were separated by a half-bordered pit and chunky deposits were observed in both cells (e.g. Fig. 7). UV absorption due to aromatic compounds was absent in the ray deposits whereas a typical lignan curve was obtained from the tracheid.

## Deposits External to Wood Cells

### *Checks*

One wood sample contained checks about 2 ... 4 cm long in the pith region; these were possibly due to frost and were occluded with whitish deposits. GLC analysis indicated this material was very largely matairesinol. On more detailed microscopic investigation several shapes of deposits were recognizable in the checks. Analysis of large chunky portions showed these to be mainly matairesinol with a small amount of conidendrin. One of the samples of a white flaky or feathery deposit was largely hydroxymatairesinol with about 3% matairesinol, other samples contained largely matairesinol. A round globular deposit in this zone was mostly conidendrin but a significant amount of the unknown 0.53 was also present. The purity of the individual deposits was remarkable since all these different shaped deposits occurred close together in the same zone.

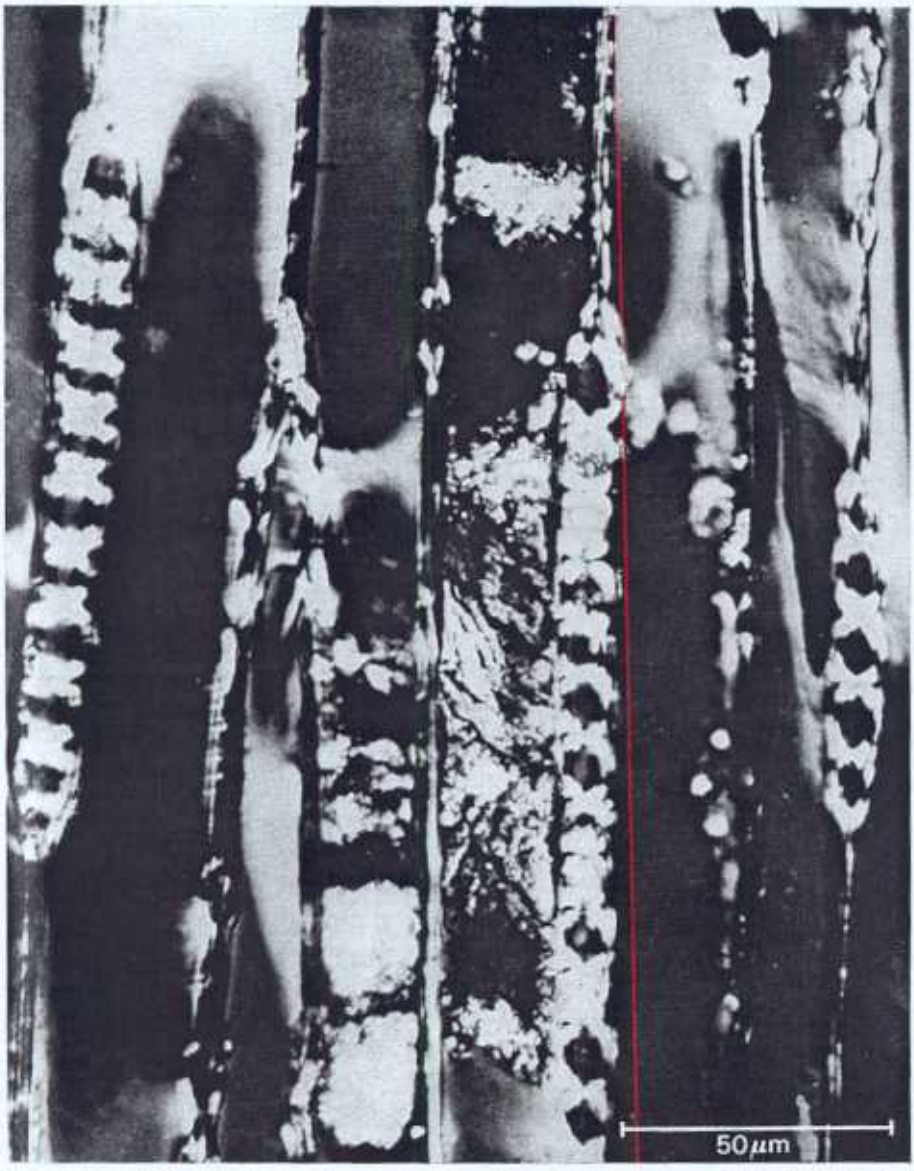



Fig. 6. Tangential-longitudinal section showing deposits of similar appearance in both longitudinal tracheid and ray parenchyma lumens. Photo taken with polarized light

*Surface films*

Some wood samples developed a whitish cast on the transverse surface when wet areas were allowed to dry. This material was scraped from the surface and on analysis by GLC found to contain large amounts of both hydroxymatairesinol and conidendrin. There were also small amounts of matairesinol and many unknown peaks which eluted prior to hydroxymatairesinol. Although the lignans

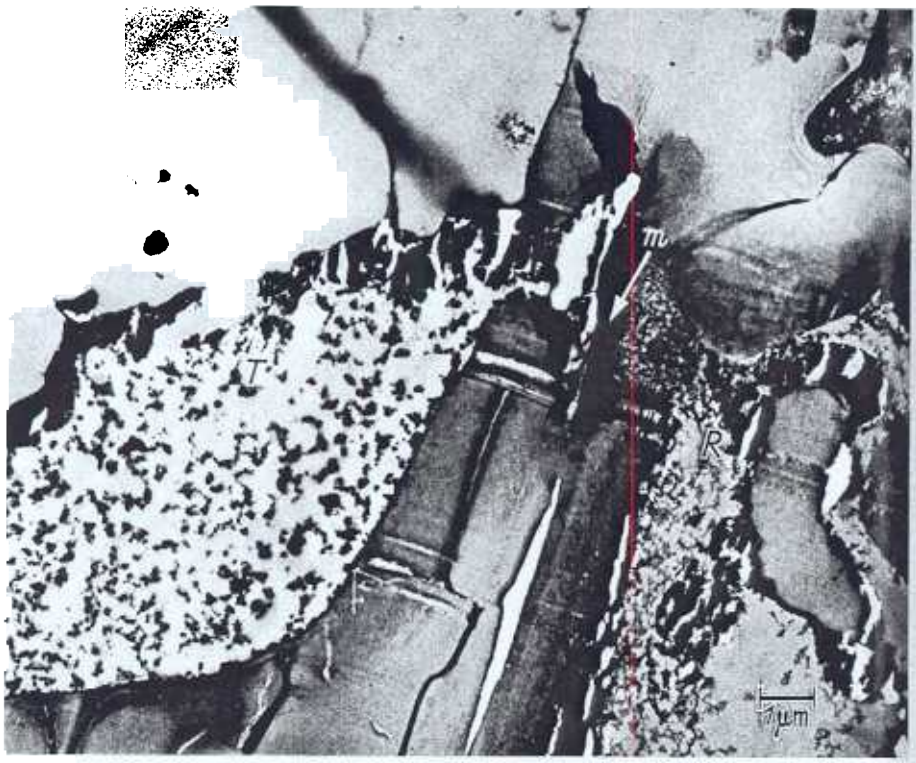



**Fig. 7.** Transverse section through a floccosoid zone illustrating occlusions in a tracheid lumen (T) and a ray cell (R). Dissimilar deposits appear to occlude the pit cavities on either side of the membrane (M). Ultra-thin section $KMnO_4$ fixed

are not very soluble in water, a white film was noticed on microscope slides where sections had been placed in water. Analysis of this white material also showed all three lignans in much the same proportion as was found for the wood sample itself. The relatively high degree of mobility of the lignans in aqueous media might be an explanation for the blockage of the pit membranes observed in the photomicrographs Fig. 5. These deposits may have arisen during the natural drying of the wood when the lignans were moved to and deposited on the pits.

## Distribution of Lignans Across Wet Wood Zones

Radial strips divided into sapwood, wet heartwood and normal heartwood were extracted with acetone and the extracts examined by thin layer chromatography. There was no apparent relationship between lignan concentration and location of wet wood zones. Both conidendrin and hydroxymatairesinol occurred in patches, and regions of high hydroxymatairesinol content could not be related to either high or low concentrations of conidendrin. Samples containing large amounts of either hydroxymatairesinol or conidendrin were found in both wet and normal heartwood zones. None of the unidentified spots observed on TLC plates appeared to be related to zones of wet heartwood.

Wet wood zones may be associated with lignans existing as very fine boundaries. To determine whether this was so, a series of small samples was taken from areas free from floccosoids. The series passed from normal heartwood through the transition zone into wet heartwood. The samples were examined by GLC and the five sets of samples, each containing several wet wood zones, failed to reveal a significant, consistent difference in lignan concentration.

## Discussion

Generally in the study of wood only a gross relationship between the presence of extractives and their biosynthesis or influence on properties has been considered. Information obtained at the cellular level will give a much more precise understanding of the origin and influence of extractives. This examination of western hemlock not only revealed the extractives composition of small samples of particular tissues but also the composition of different portions of the same cell. We have drawn the following conclusions from this study related to wood properties and biochemistry.

### Wood Properties

Deposits which largely block tracheid lumens would appear to have little influence on physical properties because of their isolated, infrequent spacing in the wood structure. Our results indicate that heartwood contained small amounts of lignans dispersed throughout the tracheids together with patches of larger amounts of lignans in a rather pure form. Lignans were found by GLC in tracheids which optical microscopy indicated to be free of deposits. Lignans lining tracheid walls were observed with the MSP and occlusions in bordered pits also gave lignan spectra. Electron microscopy indicated that the major portion of the pit encrustation and wall linings was removed by acetone extraction. It is highly probable that the pit occlusion shown in Fig. 5 is a lignan coating.

Linings on the tracheid walls should have an important bearing on such properties as liquid permeability. The absence of a relationship between lignan concentration and position of the wet-wood zone in our study does not exclude lignans from responsibility. The extent to which lignan coatings cover the pits could well be greater in the wet-wood-dry-wood transition zone and clearly further work on the morphology of the encrustation is required.

Although the lignans, especially conidendrin, are not very water soluble, they exhibited a high degree of mobility in aqueous systems. Both the tracheid walls and cut surfaces of wet wood were lined or coated with lignans. GLC analyses of films from cut surfaces indicated a general dispersal of mixed lignans. The mixed nature was in marked contrast to the more pure larger deposits found in tracheid lumens. Movement with water from points of high purity and mixing prior to deposition as surface films may explain the lack of purity of the coating materials. Preferential deposition of coatings on the pits together with the relatively high degree of mobility of the lignans suggests that the encrustation of bordered pits may be caused by movement to this site as the wood dried.

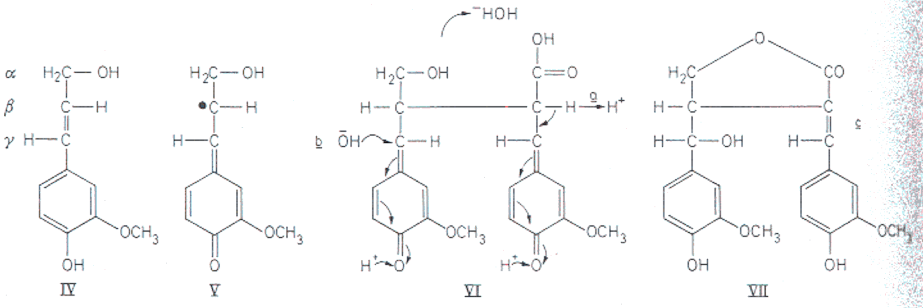
Because both tracheid walls and external surfaces were covered with lignans which have a relatively low hygroscopicity these compounds probably influence

the surface properties. Physical properties of the wood, such as wetting, should be interpreted with cognizance of lignan surface films. Although additional work is needed, it does appear that lignans are present within the cell walls. Properties such as volumetric shrinkage, which are affected by bulking of the cell wall, also may be influenced by the lignans.

## Biochemical Aspects

The cambial region of western hemlock contains catechins, leucocyanidins and polyphenols which may be precursors of lignans. The latter include coniferin, depsides of cinnamic acid derivatives, alicyclic acids and glycosides of lignan-like compounds. These compounds, except the depsides, were present in the sapwood where the major extractable components were lignans [GOLDSCHMID, HERGERT 1961]. At the heartwood boundary and in the transition zone there is a marked increase in the amounts of the lignans conidendrin, hydroxymatairesinol and matairesinol. Floccosoids, not observed in the sapwood, were found in the heartwood and in the transition zone [GOLDSCHMID, HERGERT 1961; BARTON, GARDNER 1966; BARTON 1968].

Synthetic lignin preparations demonstrate that lignin is formed by quite random oxidative coupling of $C_6$-$C_3$ units provided by coniferyl alcohol IV [HARKIN 1967]. Although neither intermediates nor enzymes involved in the biosynthesis of lignans have been isolated [NEISH 1965] there is little doubt that the lignans also arise by oxidative coupling of these $C_6$-$C_3$ units [WEINGES, SPÄNING 1967]. However by comparison with that of synthetic lignin the route to lignans is very specific. Lignan biosynthesis in western hemlock wood appears to result from: (a) equi-molar amounts of p-quinone methide radicals provided by coniferyl alcohol V and ferulic acid; (b) exclusive $\beta$-$\beta'$ coupling of these p-quinonemethide units to give VI; (c) exclusive $\alpha$-$\alpha'$ lactone ring closure with external stabilization



IV Coniferyl alcohol
V p-Quinone methide radical of coniferyl alcohol
VI $\beta$-$\beta'$ Coupling of coniferyl alcohol and ferulic acid radicals and two known routes to stabilization
VII Possible intermediate which after reduction at (c) would yield hydroxymatairesinol

by loss of proton at "a" in VI, addition of hydroxyl at "b", leading to a product such as VII [compare HARKIN 1967]; (d) strict control of stereochemistry. It appears that lignan biosynthesis unlike that for lignin is strongly enzymically controlled because of the exclusive $\beta$-$\beta'$ coupling and $\alpha$-$\alpha'$ lactone ring closure and

the stereochemistry at the two $\beta$ carbons. Reduction of compound VII would yield hydroxymatairesinol which could form conidendrin as occurs in vitro. Matairesinol could conceivably result from stabilization of both sides of the dimer VI by loss of $\beta$ protons followed by reduction. In this study, the amounts of hydroxymatairesinol and conidendrin have been observed to vary markedly from cell to cell. Accordingly conidendrin, if it is formed from hydroxymatairesinol, probably does not result from a slow change over long periods of exposure to the low pH conditions in heartwood. Conidendrin is more likely formed rapidly by metabolic processes and we have found a relatively selective formation of the three main lignans I, II and III in different locations. We have discussed the above aspects of lignan synthesis for the sole reason to draw attention to the complex enzyme system that must exist. This system enables one class of lignans, and indeed one optically active member of this class, to be deposited almost exclusively in individual tracheids.

The site of biosynthesis of heartwood extractives has received much attention and there is a large body of evidence supporting the view that polyphenolic extractives are formed in situ [HILLIS 1968]. However some workers have postulated that heartwood extractives are formed elsewhere and translocated to the heartwood boundary [e.g. HERGERT, GOLDSCHMID 1958; STEWART 1966]. The latter views have been specifically expressed for western hemlock by BARTON and DANIELS [1969] and GOLDSCHMID and HERGERT [1961]. The intermediates have been considered to be the glucosides of the lignan-like compounds or the relatively water soluble hydroxymatairesinol [GOLDSCHMID, HERGERT 1961; BARTON, GARDNER 1966]. Hydroxymatairesinol [GOLDSCHMID, HERGERT 1961] and a synthetic conidendrin glucoside [BARTON, DANIELS 1969] have been readily converted to conidendrin in vitro.

If translocation of the above intermediates took place they should be detected in the rays, particularly in the heartwood rays, where they could accumulate. No aromatic absorption was observed in the rays with the MSP apart from the occasional effect due to lignin in the cell walls. This was so even where deposits were only separated by a half bordered pit pair and the tracheid side contained large amounts of lignans. GLC studies also indicated that rays were devoid of lignans. Furthermore, translocation would probably result in a composition and amount of extractives that was relatively uniform in tangential sections of the heartwood. However, marked differences in both amount and composition of the lignans in individual and small groups of tracheids were found by GLC. Those present can be very largely conidendrin or hydroxymatairesinol or an unknown component (0.53). Although not strictly analogous to heartwood formation, the discrete formation of different lignans in the check also suggested that control of lignan synthesis was close to the site of lignan accumulation. Deposits in the check were the only samples that contained matairesinol as the dominant lignan. Such a marked shift in composition would not be expected if the lignans were synthesized in areas remote from the check. If translocation of cambial extractives in their original proportions occurs, then a large amount of catechin and leucocyanidin polymers would be found in the heartwood. This has not been observed [BARTON, GARDNER 1966]. With these data and the recently determined distribution of lignans in *Thuja plicata* Donn [SWAN, JIANG, GARDNER 1969] we conclude

that it is improbable the heartwood extractives are synthesized in the cambium and outer sapwood and translocated to the transition zone.

Our results indicate that the lignans may be synthesized at the sapwood-heartwood boundary in the ray cells contiguous with the occluded tracheids. The lack of significant amounts of aromatic materials in the ray cells suggests that the lignans arise from primary metabolites and the phenolic pool size in the ray is small. Enzyme activity has been shown [BAUCH, LIESE, SCHOLZ 1968; THOMAS 1968] to be present on the pit margo and activity at this location may play a part in lignan formation. It seems more probable, however, that they may be synthesized in the ray cytoplasm adjacent to the pit and then forced into the tracheids through the pit membrane. A similar occurrence takes place when tyloses are formed in the vessels adjacent to ray cells [MEYER, CÔTÉ 1968] or in the formation of "gums" when the pit aperture is less than a certain size [CHATTAWAY 1949]. An alteration of the biochemical conditions of individual ray cells could result in different lignans in adjacent tracheids. Examination of the cytological conditions in ray cells adjacent to tracheids undergoing floccosoid formation may provide the necessary evidence to clarify the situation.

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R. L. KRAHMER*, R. W. HEMINGWAY and W. E. HILLIS
Physiology and Microstructure Section
Division of Forest Products, C. S. I. R. O.
P. O. Box 310
South Melbourne, Australia

* On sabbatical leave from Oregon State University U. S. A.