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# THE LIGNANS OF *THUJA PLICATA* AND THE SAPWOOD–HEARTWOOD TRANSFORMATION*

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**Abstract**—A chromatographic study was made of the polyphenols present in the sapwood and heartwood extractives from western red cedar. Quantitative analyses for plicatic acid, plicatin, thujaplicatins and thujaplicatin methyl ethers were performed on sections from two trees. The results were consistent with the formation of these lignans at the heartwood boundary *in situ* and with continuation of their chemical transformation well within the outer heartwood by an hydroxylation process. The thujaplicins in the heartwood probably account for the presence of large amounts of the thujaplicatins, compared to the thujaplicatin methyl ethers, by their inhibition of the further methylation of the monomethyl pyrogallol ring.

## INTRODUCTION

THE HEARTWOOD extractives of western red cedar (*Thuja plicata* Donn) have been studied for many years. Among the first components to be identified were the thujaplicins (I).[1] Later the lignans plicatic acid (II), and its lactone (III) and the lignans of the thujaplicatin series (IV), and the thujaplicatin methyl ether (T.M.E.) series (V) were isolated and characterized.[2–8] The reasons for studying these lignans are the unusual structures and the large amounts of them found in the heartwood.[9] Plicatic acid (II) was the first lignan acid found in nature. It is a very strong acid because of its highly hydroxylated side-chain—another unique feature, and the pendant ring is the unusual monomethyl pyrogallol group. Also, the thujaplicatin methyl ethers (T.M.E.) (V) are the first syringyl derivatives (pendant ring) found in a softwood and the first lignans with both guaiacyl and syringyl rings. Finally, the thujaplicins (I) are tropolone derivatives and these are not widely distributed outside the Cupressaceae.

The questions of how these components are biosynthesized and of the site of their formation are intriguing. In this work, as in all others on lignans to date, there was no evidence of intermediates. As Neish has pointed out, in reference to lignan biosynthesis, there have been

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[1] H. ERDTMAN and J. GRIPENBERG, *Acta Chem. Scand.* **2**, 625 (1948).

[2] J. A. F. GARDNER, G. M. BARTON and H. MACLEAN, *Can. J. Chem.* **37**, 1703 (1959).

[3] J. A. F. GARDNER, B. F. MACDONALD and H. MACLEAN, *Can. J. Chem.* **38**, 2387 (1960).

[4] J. A. F. GARDNER, E. P. SWAN, S. A. SUTHERLAND and H. MACLEAN, *Can. J. Chem.* **44**, 52 (1966).

[5] H. MACLEAN and K. MURAKAMI, *Can. J. Chem.* **44**, 1541 (1966).

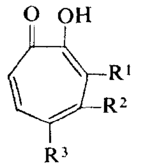
[6] H. MACLEAN and K. MURAKAMI, *Can. J. Chem.* **44**, 1827 (1966).

[7] H. MACLEAN and K. MURAKAMI, *Can. J. Chem.* **45**, 305 (1967).

[8] H. MACLEAN and B. F. MACDONALD, *Can. J. Chem.* **45**, 739 (1967).

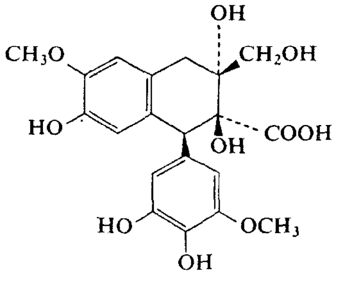
[9] H. MACLEAN and J. A. F. GARDNER, *Forest Prod. J.* **6**, 510 (1956).

no intermediates isolated, nor are there any known enzymes catalysing the observed $\beta$–$\beta'$ coupling of two $C_6$–$C_3$ units; all that can be said is that coupling is not an oxidative pathway through quinone methides as in lignin.[10] By analogy with work performed on microflora, however, the probable pathways to these compounds can be described. This paper reports analytical work which shows the lignans to be present in the sapwood of *T. plicata*, although in low concentration, and determines their distribution in the sapwood and outer heartwood.

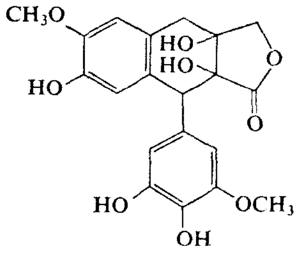


$R^2 = R^3 = H$, $R^1$ = isopropyl-$\alpha$ isomer
$R^1 = R^3 = H$, $R^2$ = isopropyl-$\beta$ isomer
$R^1 = R^2 = H$, $R^3$ = isopropyl-$\gamma$ isomer

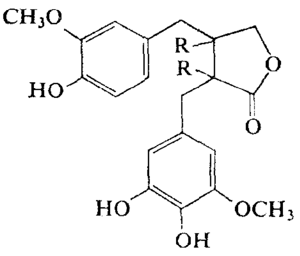
(I) Thujaplicins



(II) Plicatic Acid

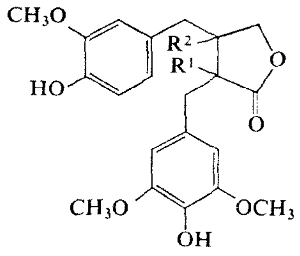


(III) Plicatin



(IV) Thujaplicatins

(a) R = H
(b) R = OH



(V) Thujaplicatin Methyl Ethers (T.M.E.)

(a) $R^1 = R^2 = H$
(b) $R^1 = OH$, $R^2 = H$
(c) $R^1 = R^2 = OH$

## RESULTS AND DISCUSSION

### *Comparison of the Sapwood and Heartwood Phenolic Extractives*

A two-dimensional paper chromatogram of the heartwood extractives from western red cedar gives five distinct phenolic spots. The various spots have been identified as plicatic acid (II), its lactone (III), the thujaplicatins (IV), mixture of T.M.E.s (V), and thujaplicins (I).

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[10] A. C. NEISH, in *Plant Biochemistry* (edited by J. BONNER and J. E. VARNER), pp. 585–594, Academic Press, New York (1965).

In comparison, the corresponding chromatogram from the extractives of western red cedar sapwood gives six spots, the four central ones being catechins and leucoanthocyanins which were readily oxidized by treatment of the extracts with air to yield insoluble tannins. The chromatogram of the oxidized extract only showed spots corresponding to thujaplicatins (IV) and the mixture of T.M.E.s (V). Unfortunately, no paper-chromatographic system could be found which completely separated these remaining closely related compounds. They could be separated, however, by TLC on silica gel G using benzene–ethanol (9:1). Using this separation technique, T.M.E. itself (Va) was shown to be the major sapwood lignan, but its derivatives as well as thujaplicatin (IVa) and its derivatives were also present. Plicatic acid (II) and its lactone (III) did not develop with this thin-layer chromatography system. Their presence in only minute amounts was inferred from paper chromatographic examination of the extractives. Since paper chromatograms of the sapwood extractives were unchanged after treatment with emulsin it was concluded that no appreciable amounts of phenolic glucosides were present in the sapwood extract.

The discovery of the lignans (II) to (V) inclusive, in the sapwood of western red cedar, albeit in amounts 100 times smaller than the heartwood, was interesting and encouraging. For instance, it meant that the biosynthetic pathways leading to the lignans could be studied (at a later date) by radiochemical techniques applied to sapwood. It is clear that all sapwood components must be isolated and characterized, even though they may be present in only a few parts per million. This present work has shown only the end-products of the lignan metabolic pool. Unknown intermediates may be present in much smaller amounts. One original aim of this research was to identify the intermediary metabolites (perhaps present as glycosides). The results show that the search for these elusive compounds will have to start at the cambium; either by *in vivo* experiments, or perhaps from *in vitro* experiments with cambial scrapings.

### *Extractives Distribution*

In order to determine the distribution of the lignans within the tree, as had been done by MacLean and Gardner for the thujaplicins,[9] a search was made for suitable methods of analysis. A paper chromatographic method of analysis was chosen in preference to TLC or GLC techniques. As noted above, the paper chromatographic method did not separate individual members of the thujaplicatins (IV) and T.M.E.s (V) families and they were therefore determined as groups. Plicatic acid (II) and plicatin (III) were determined as such. The thujaplicins (I) were also determined by the usual colorimetric method.[11] Table 1 shows the distribution of these compounds across two trees. Note that the ring numbering begins at the cambium and goes towards the pith, rather than beginning at the pith as usual.

Table 1 shows a large surge in chemical activity at the sapwood–heartwood transition, the concentrations of most compounds increasing by at least 100 times. However, the T.M.E.s (V) highest in the sapwood (in Tree 1 but not Tree 2) only increase by about 10 times. The formation of T.M.E.s (V), in both the sapwood and heartwood, was presumably via enzymatic *O*-methylation of thujaplicatins (IV). However, the much greater increase in IV content in the heartwood relative to V, together with the presence of appreciable amounts of thujaplicins (I) in the newly formed heartwood, suggested that I was inhibiting some *O*-methylation. The result was a much greater ratio of IV (and hence II and III as discussed below) to V in the heartwood compared to the sapwood. The average ratio of catechol-group

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[11] H. MacLean and J. A. F. Gardner, *Anal. Chem.* **28**, 509 (1956).

containing (II, III, IV) to the methyl ether V was 0·7 and 6 for the sapwood and heartwood of Tree 1, and 5 and 10 for the same in Tree 2. The inhibiting effect of the thujaplicins on *O*-methylations in animal systems has been studied.[12]

Table 1 also showed that the maximum lignan content was not at the coloured, visible sapwood–heartwood boundary, but occurred fifty to one hundred annual increments past it towards the pith. Thus the biochemical transformations were operative well into the heartwood zones. The formation of different amounts of each extractive in the two trees can be explained by changes in metabolism (more hydroxylating, less methylating) with ageing. Thus Table 1 suggests that the lignans were formed in the sequence thujaplicatin to plicatin to plicatic acid. This could take place by the pathway illustrated in Fig. 1, the hydroxylations occur first at the $\beta$-positions, then at one of the $\alpha$ to form the tetralin ring, and then at the appropriate $\gamma$ to form plicatic acid itself. If the hydroxylation pattern is correct we might expect to isolate the isomer of plicatin (VI) formed by hydroxylation of the other $\alpha$-position (Fig. 2).

TABLE 1. EXTRACTIVES VARIATION IN THE RADIAL DIRECTION

| | % yields, oven-dried wood basis | | | | |
|---|---|---|---|---|---|
| Ring No.* | I | II | III | IV | V |
| Tree 1 | | | | | |
| 1 | | 0·01 | —‡ | 0·001 | 0·06 |
| 2 | | 0·01 | — | 0·002 | 0·06 |
| 3 | | 0·01 | — | 0·002 | 0·05 |
| 4 | | 0·01 | — | 0·001 | 0·02 |
| 5 | | 0·01 | 0·01 | 0·004 | 0·03 |
| 6 | | 0·01 | 0·05 | 0·09 | 0·27 |
| 7† | 0·01 | 0·02 | 0·30 | 0·23 | 0·77 |
| 8† | 0·12 | 0·02 | 0·67 | 0·83 | 0·43 |
| 9–10 | 0·17 | 0·06 | 1·38 | 1·65 | 0·58 |
| 11–12 | | 0·08 | 2·34 | 1·58 | 0·55 |
| 13–14 | | 0·33 | 3·05 | 1·06 | 0·53 |
| 15–16 | 0·19 | 0·59 | 2·89 | 0·52 | 0·55 |
| 17–18 | | 1·04 | 2·59 | 0·47 | 0·64 |
| 19–20 | 0·31 | 1·21 | 3·07 | 0·75 | 1·06 |
| 21–22 | | 1·23 | 2·49 | 0·83 | 0·57 |
| 30 | 0·34 | 1·99 | 2·10 | 1·87 | 0·74 |
| 45 | | 0·44 | 1·70 | 1·00 | 1·03 |
| 65 | | 0·33 | 0·67 | 0·72 | 0·29 |
| Tree 2 | | | | | |
| 1 | | 0·07 | 0·03 | 0·04 | 0·03 |
| 5 | | 0·07 | 0·02 | 0·03 | 0·02 |
| 10 | | 0·08 | 0·02 | 0·04 | 0·03 |
| 16† | | 0·16 | 0·13 | 0·26 | 0·04 |
| 17† | | 0·70 | 1·63 | 4·47 | 1·21 |
| 50 | | 1·32 | 4·54 | 3·21 | 1·44 |
| 75 | | 2·81 | 4·46 | 3·43 | 1·53 |
| 100 | | 2·30 | 3·07 | 2·84 | 0·96 |

* The ring numbering began at the cambium and went towards the pith.
† Location of the visible sapwood–heartwood boundary.
‡ None was observed on the paper chromatogram.

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[12] B. BELLEAU and J. BURBA, *Biochem. Biophys. Acta* **54**, 195 (1961).

Another possible hydroxylating sequence in the heartwood is suggested by the recent discovery of 4-isopropylytropone in western red cedar heartwood.[13] This substance could give rise to the thujaplicins by hydroxylation and they in turn (β or γ isomers) to the co-occurring β-thujaplicinol[14] (Fig. 3). The hydroxylation mechanism is perhaps by the direct reaction of

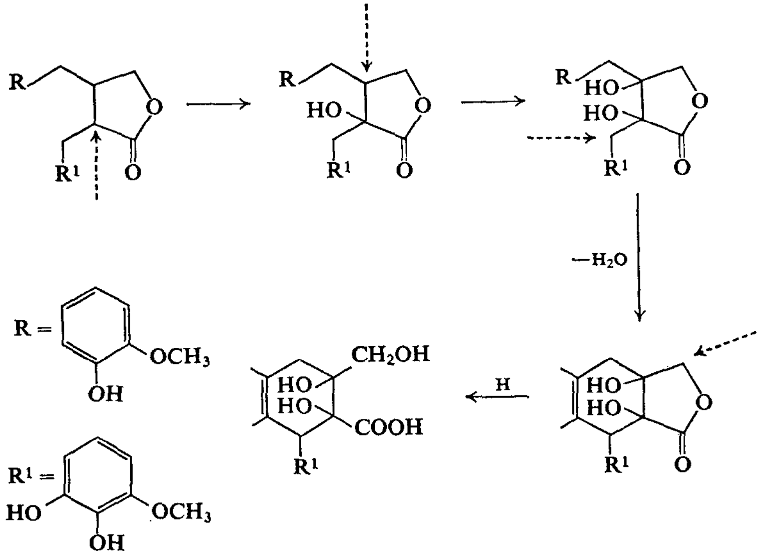
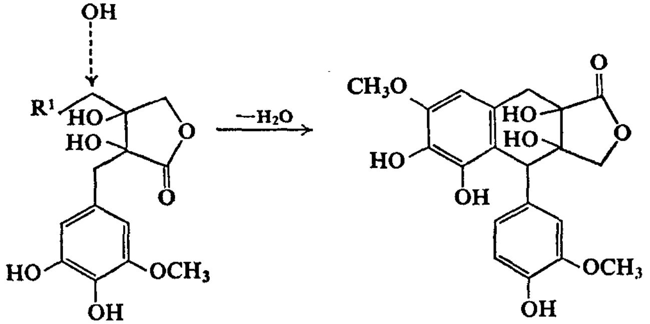


Fig. 1. Proposed hydroxylation pathway in the biosynthesis of plicatic acid. Dashed arrow indicates hydroxyl group introduction.



Isoplicatin

(VI)

Fig. 2. Proposed formation of the isomer of III.

the precursor with oxygen as suggested by Frey-Wyssling and Bosshard[15] who studied the cytology of the ray cells in sapwood and heartwood in twelve species. They concluded that the transition between the sapwood and heartwood was characterized by a semi-anaerobic

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[13] Y. Hirose and T. Nakatsuka, *J. Japan Wood Res. Soc.* **13**, 123 (1967).

[14] J. A. F. Gardner, G. M. Barton and H. MacLean, *Can. J. Chem.* **35**, 1039 (1957).

[15] A. Frey-Wyssling and H. Bosshard, *Holzforsch.* **13**, 129 (1959).

metabolism. In the heartwood, a slow oxidation and polymerization of extractives occurred by direct reaction with oxygen.

Such oxidations account for the observed decrease in extractives in mature heartwood. The rapid decrease in content of plicatic acid towards the pith after its maximum content is reached is attributed either to the lesser ability of the tree when younger to synthesize it, or to its self-polymerization due to its strongly acidic nature,[2] or to further oxidation of it to other lignans which are presently being studied at the Vancouver Forest Products Laboratory. Similarly, the flavonoid derivatives shown in the sapwood extract could be considered part of the metabolic pool, and they are probably polymerized in the heartwood. This polymerization produces tannins which, because of the loss of water from the heartwood,[16] are precipitated in the cells giving the characteristic colour of the heartwood. This hypothesis is difficult to test because there is no simple way of removing the wood-colouring matter from extractive-free wood.

Krahmer and Côté,[17] who also worked on *Thuja plicata,* showed photographs from electron microscopy of the tracheids. Those tracheids from the heartwood were heavily encrusted with extractives, those from the sapwood were clean. The extractives were probably carried to the heartwood via the ray cells. Also, present theories[18] on heartwood formation state that polyphenols are synthesized from carbohydrate precursors. Thus Hillis and Hasegawa, in experiments with *U*-$C^{14}$-D-glucose,[19] showed polyphenols in *Eucalyptus sieberiana* kino were synthesized from the carbohydrate. This research substantiated this theory becase of the large ratio of catechol-group containing IV to the methyl ether V in the newly formed heartwood, compared to the sapwood, discussed above. Only if the ratio of all polyphenols remained constant across the sapwood to the heartwood could the translocation theories be invoked, but this was not the case. This assumed that the solubility of these lignans was the same; which was true, II–V were all water-soluble.

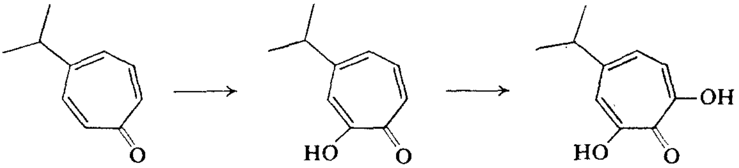

FIG. 3. POSSIBLE HYDROXYLATION SEQUENCE OF OTHER EXTRACTIVES IN WESTERN RED CEDAR.

## CONCLUSIONS

The major proportion of the lignans of western red cedar are formed *in situ* at the sapwood–heartwood boundary from precursors because the analytical results from the two trees showed a rapid increase, together with a change in proportions, of the lignans at the boundary (provided the lignans and precursors are transported at the same rate). The transformation of the lignans is not complete at the visible boundary but continues into the heartwood for many years. This process is probably a hydroxylating one, and the production of plicatic

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[16] C. M. STEWART, *Nature* **214**, 138 (1967).

[17] R. L. KRAHMER and W. A. CÔTÉ, JR., *Tappi* **46**, 42 (1963).

[18] W. E. HILLIS, *Wood Extractives and their Significance to the Pulp and Paper Industries*, pp. 107–123, Academic Press, New York (1962).

[19] W. E. HILLIS and M. HASEGAWA, *Phytochem.* **2**, 195 (1963).

acid is thought to occur by the sequence thujaplicatin to dihydroxythujaplicatin to plicatin to plicatic acid. The presence of the enzyme inhibitors, the thujaplicins, is thought to be responsible for the production of thujaplicatin rather than thujaplicatin methyl ether.

## MATERIALS

The work described here was performed on sections from two trees. Tree 1 was a western red cedar (*Thuja plicata* Donn), 90 years old, 12 rings per in., growing at Road E, site index 100–135 in the University of B.C. Forest at Haney, B.C. The heartwood was a light-straw colour throughout with no visible signs of fungal attack. The sapwood–heartwood boundary (between rings 5–7 from cambium) was obtained by visual inspection. Tree 2 was from Ucluelet Inlet, B.C. In excess of 400 years old, it had 22 rings per in., and the sapwood–heartwood boundary was about rings 16 and 17. Of course, the visible boundary wandered between these rings and was not confined to the annual increment only. The samples were kept at about 0° in the dark and only small sections were taken as needed. Extractions were performed in a soxhlet with MeOH; using acetone gave lower yields of II. Reagent grade solvents were used throughout without further purification.

## METHODS

### 1. *Paper Chromatography*

Paper chromatography was performed on Whatman No. 1 paper. For the two-dimensional chromatographs, the same solvent systems and detecting reagents were used as were used in the study of western red cedar phloem extractives.[20] For the analyses of the heartwood lignans (II–V) the solvent system used was butanol, acetic acid, dilute molybdic acid on molybdic acid-impregnated paper.[21] Unfortunately, T.M.E. (V) could not be determined on the latter system because it ran too close to the solvent front. The solvent used was 2% acetic acid, in which both IV and V had $R_f \simeq 0{\cdot}5$. The amount of IV determined on the molybdic acid-impregnated paper system above was substracted from the total $R_f \simeq 0{\cdot}5$ (dilute acid solvent) to give the V content. In all cases, standards were spotted simultaneously and the colorimetric-analytical method of Keith *et al.*[22] was used. Other methods of analyses which failed were densitometry on thin-layer plates and gas chromatography of the trimethylsilyl-derivatives.

### 2. *Thin-Layer Chromatography*

This was performed on silica gel G plates, the developing solvent was benzene–ethanol (9:1) and the detecting spray was concentrated nitric and sulphuric acids (1:1). The $R_f$ characteristics of the heartwood lignans have been described.[4–6]

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[20] E. P. SWAN, *Tappi* **46**, 245 (1963).

[21] J. B. PRIDHAM, *J. Chromatog.* **2**, 605 (1959).

[22] R. W. KEITH, D. LE TOURNEAU and D. MAHLUM, *J. Chromatog.* **1**, 534 (1958).