*Reprint from Appita, Vol. 24, No. 6*

*Commonwealth of Australia, Commonwealth Scientific and Industrial Research Organization. Reprinted from "Appita" Volume 24, No. 6, Pages No's. 439-443, May 1971. D.F.P. Reprint No. 853.*

# CHANGES IN FATS AND RESINS OF PINUS RADIATA ASSOCIATED WITH HEARTWOOD FORMATION

R. W. HEMINGWAY* and W. E. HILLIS**

**SUMMARY**

In an analysis of Australian grown *P. radiata* marked changes were found in the relative proportions and compositions of the resin acids, fatty acids, and fatty acid esters associated with heartwood formation. While the proportion of resin acids increased substantially in the heartwood, there was little change in resin acid composition from outer sapwood to inner heartwood. Extracts from wood of three large trees and samples of oleoresin from three small trees contained resin acids of similar composition.

Fatty acids were found in only trace proportions in the sapwood but were more abundant in the heartwood. The composition of sapwood fatty acids differed from that in the heartwood and from that of the sapwood fatty acid esters. Large reductions in the proportions, and a marked change in the composition, of fatty acid esters occurred from outer sapwood to heartwood.

*Pinus radiata* is a major species employed for plantation of softwood timber in Australia, New Zealand, Chile and South Africa. Until recently, little attention has been paid to the fats and resins of *P. radiata* and even less to changes in these compounds during heartwood formation. Buckland and co-workers (1) and McDonald and Porter (2) found that the resin acid concentration was highest in the inner heartwood at the base of the tree and that there was less fatty acid ester than fatty acid in both sapwood and heartwood. After completion of the work reported in this paper Porter (3) published results of an additional study in which he found only small amounts of fatty acids in both sapwood and heartwood from a tree grown in New Zealand. The resin acids were concentrated in the heartwood while fatty acid esters were concentrated in the sapwood. The resin acid composition found by Porter is similar to that found by Lawrence (4) for South African grown *P. radiata*. Hansen (5) has also examined the composition of the total of fatty acids and fatty acid esters in wood from a *P. radiata* tree grown in New Zealand and reported a different composition of the fatty acids from that obtained by Porter.

In order to obtain an understanding of pitch problems encountered in the production of paper from Australian grown *P. radiata* bisulphite pulps (6) an estimate was sought of the proportions and compositions of the resinous material in fresh wood. The amount of heartwood in pine logs is variable and its inclusion in the raw material for pulping may be avoided by selection of suitable harvesting schedules. It was therefore of particular interest to examine the changes in the resinous extractives associated with heartwood formation. This paper presents the findings on the distribution and composition of the resin acids, fatty acids and fatty acid esters with particular reference to heartwood formation.

## EXPERIMENTAL

### Wood samples

Three trees were sampled to determine the amounts and compositions of fatty acids, resin acids, and fatty acid esters in *P. radiata* wood. Tree 1 was about 50 years old, of 14 in diameter, with a heartwood zone 5 in diameter. This tree was taken from a plantation near Mt. Macedon, Victoria. Trees 2 and 3 were obtained from a plantation near Penola, South Australia. Tree 2 was 30 years old, 8 in diameter, with a heartwood zone 4 in diameter. Tree 3 was 30 years old, of 4 in diameter, with a heartwood 1 in diameter. All bolts were frozen within 24 hours after felling the trees and stored at —15°C prior to analysis. The wood from Trees 1 and 2 was divided into outer and inner sapwood and outer and inner heartwood, and that from Tree 3 was only divided into sapwood and heartwood. The wood was split while frozen into matchstick sized samples, freeze dried for two days, ground in a Wiley mill to pass a 40 mesh screen, and extracted with either 40 to 60°C petroleum or acetone for at least 8 hrs. The extract was dried in vacuo at less than

* Research Scientist, Division of Forest Products, CSIRO. Full Member, Appita.

** Senior Principal Research Scientist, Division of Forest Products, CSIRO. Full Member, Appita.

30°C. Three greenhouse trees were also selected to obtain samples of oleoresin.

**Analysis of wood extracts**

Samples of the acetone soluble material (30 mg) were immediately separated into acidic and neutral fractions on diethylaminoethyl (DEAE) Sephadex columns prepared according to Zinkel and Rowe (7). The neutral fraction was eluted with 60 ml of diethyl ether-methanol-water (89/10/1 by volume) and acids with 175 ml of $CO_2$ saturated diethyl ether-methanol (90/10 v/v). The neutral fraction was saponified by heating it under reflux in 2 N methanolic KOH for eight hrs and the saponifiable fatty acids were extracted into diethyl ether from acidified solutions. The acidic (fatty and resin acids) and esterified fatty acid (saponifiable fatty acids) fractions were methylated with diazomethane in diethyl ether and then dissolved in 5.0 ml of acetone.

Samples of these two solutions (5.0 lambda aliquots) were immediately examined by gas-liquid chromatography (GLC) using a Varian 2100 chromatograph on 2 to 3mm ID glass columns packed with six per cent diethyleneglycol succinate coated on 80 to 100 mesh chromosorb W treated with dimethylchlorosilane. The helium carrier gas flow rate was 40 ml/min. The oven temperature used was 160°C for analysis of fatty acid methyl esters from the fatty and resin acid fraction and the esterified fatty acid fraction, and 185°C for analysis of the resin acid methyl esters from the fatty and resin acid fraction.

The proportions of individual or groups of compounds were determined by comparing GLC peak areas obtained by measurement of the product of peak width at half height and peak height. Previous calibration of the fatty acid methyl esters (8) and measurement of peak areas obtained by injecting widely varying amounts of the above fractions, gave a linear relationship between the amount of material injected and the GLC peak area. The difference in detector response for each compound was not measured so results must be considered as relative rather than as absolute quantities. Results obtained with the above analytical method were compared with those obtained by gravimetric methods using large resin samples and the two methods gave very similar results (6). Preparation of the compounds for analysis by extraction with either petroleum or acetone and separation of the acidic and neutral fractions using DEAE Sephadex columns did not cause significant oxidation or isomerization of the sensitive resin acids. In some cases, the resin acids were separated as their cyclohexylamine salts and GLC analyses of the regenerated resin acids were compared with results from the methylated whole resin samples or samples separated on DEAE Sephadex columns. Very similar resin acid compositions were found.

**Analyses of oleoresin**

Three young *P. radiata* trees (Trees 4, 5 and 6) about 7 ft high were moved to the laboratory, an incision was made in the stem and a small amount of oleoresin methylated immediately with diazomethane. A larger oleoresin sample was collected and the resin acids separated using their cyclohexylamine salts. The regenerated resin acids were methylated and analysed by GLC. There was no significant difference between the resin acid composition of oleoresin prepared in these two ways.

## RESULTS AND DISCUSSION

**General composition**

The amounts of petroleum-soluble extractives, together with the relative proportions of fatty acids, resin acids, and fatty acid esters found in the wood samples, are shown in Table 1. The figures in Table 1 were converted to a relative amount of each fraction per gram of wood (Table 2). The proportion of unsaponifiables in the petroleum extracts was assumed to be constant. The petroleum solubility was higher in the inner heartwood than in the outer heartwood or in the sapwood (Table 1). The higher percentage of petroleum extractives in heartwood was accounted for by larger proportions of resin acids (Tables 1 and 2). In the sapwood of the three trees, the resin acids comprised about 50 per cent of the petroleum solubles, while the proportion of resin acids was an average of 94 per cent in the inner heartwood of Trees 1 and 2 (Table 1).

Trees 2 and 3 were planted in the same year but Tree 3 was suppressed. The proportion of resin acids in the heartwood of Tree 3 was low (77 per cent) compared with that in the heartwood of the more vigorous Trees 1 and 2 (88 per cent). The relatively low resin acid proportion in the heartwood of Tree 3 suggests that the resin acid content of heartwood may be related to the age of the heartwood zone or to the vigour of the tree, and that not all trees contain large proportions of resin acids in the wood near the pith.

The proportion of fatty acids present in the sapwood was very low, the average for the three trees was less than 0.5 per cent of the petroleum solubles (Table 1) or about 1.4 per cent of the total of fatty acids and fatty acid esters. The proportion of fatty acids reported in other tree species is much higher, ranging from 10 to about 50 per cent of the total of fatty acids and fatty acid esters (9). Variations in the proportions of fatty acids and fatty acid esters are related to the methods of sample preparation because of rapid hydrolysis of the fatty acid esters after felling the trees (9)(10). In comparison with sapwood, the proportion of fatty acids was much higher in the heartwood, being an average of 2.5 per cent of the petroleum extract or about 20 per cent of the total of fatty acids and fatty acid esters. This suggests that considerable hydrolysis of the fatty acid esters to free fatty acids occurs during heartwood formation. The relative amount of fatty acid esters decreased from outer to inner sapwood and further decreased with the formation of heartwood (Table 2). An average of 42 per cent of the fatty acid esters found in outer sapwood was present in the outer heartwood. Only an average of 11 per cent of the 58 per cent decrease in fatty acid esters was accounted for by an increase in the fatty acid fraction (Table 2). These results suggest that there was considerable metabolism of the fatty acid esters in the sapwood and on the formation of heartwood. Within the heartwood there was also less fatty acid ester in the inner portions than in the outer heartwood. Some, but not all, of the lower fatty acid ester content of inner heartwood was accounted for by higher proportions of the fatty acid fraction (Table 2).

**TABLE 1**

Amount and composition of petroleum solubles in *P. radiata* wood

| Sample* | Petroleum solubles | | | |
|---|---|---|---|---|
| | % of wood | % Resin acids* | % Fatty acids* | % Fatty acid esters* |
| **Tree 1** | | | | |
| Outer sap | 1.02 | 59.1 | 0.20 | 40.7 |
| Inner sap | 0.82 | 58.2 | 0.20 | 41.5 |
| Outer heart | 1.62 | 82.5 | 1.50 | 16.0 |
| Inner heart | 3.65 | 97.0 | 0.50 | 2.5 |
| **Tree 2** | | | | |
| Outer sap | 1.87 | 46.4 | 0.60 | 53.0 |
| Inner sap | 1.34 | 56.0 | 0.70 | 44.0 |
| Outer heart | 2.09 | 82.4 | 3.98 | 12.5 |
| Inner heart | 3.79 | 90.9 | 3.62 | 5.0 |
| **Tree 3** | | | | |
| Sapwood | 1.60 | 50.0 | 0.60 | 49.5 |
| Heartwood | 1.74 | 77.4 | 2.74 | 19.9 |

* Per cent of the total GLC peak areas of individual fatty acids, resin acids and fatty acid esters.

**TABLE 2**

Proportions of fatty acids, resin acids, and fatty acid esters per unit weight of *P. radiata* wood

| Sample No. | Resin acid* gram wood | Fatty acid* gram wood | Fatty acid ester* gram wood |
|---|---|---|---|
| **Tree 1** | | | |
| Outer sap | 60 | 0.20 | 42 |
| Inner sap | 48 | 0.16 | 34 |
| Outer heart | 134 | 2.4 | 26 |
| Inner heart | 354 | 1.8 | 9 |
| **Tree 2** | | | |
| Outer sap | 87 | 1.1 | 99 |
| Inner sap | 75 | 0.94 | 59 |
| Outer heart | 172 | 8.3 | 26 |
| Inner heart | 345 | 14 | 19 |
| **Tree 3** | | | |
| Sapwood | 80 | 0.96 | 79 |
| Heartwood | 135 | 4.7 | 35 |

* Petroleum solubility times the per cent of GLC peak area in Table 1.

**Composition of resin acids**

The resin acid composition found in the wood samples examined (Table 3) agreed with the composition found by Porter (3) and Lawrence (4). In comparison with sapwood, there was a small but significant decrease in the proportion of levopimaric plus palustric acids and an increase in the proportion of abietic acid in the heartwood samples. Considering the labile nature of the resin acids present, and their storage in the inner wood for up to 50 years, it is remarkable that there was no major change in the resin acid composition from outer sapwood to inner heartwood.

Because of the instability of the resin acids, there was concern that abietic and dehydroabietic acids found in the wood samples may have formed during sample preparation. Oleoresins can be analysed without extensive sample preparation. The results of analyses of exuded oleoresin (Table 4) from the three small green house trees (Trees 4, 5, and 6) suggested that, although a small amount of isomerization and oxidation may have taken place, the results obtained in the analyses of the extracted wood resins were probably close to the actual compo-

sitions. Analyses of the wood resins from the acetone extract, cyclohexylamine salt precipitates from this extract, and from a sample separated on DEAE Sephadex all gave a similar percentage of pimaric acid in the wood. Analyses of resin acids obtained from oleoresin by separation of cyclohexylamine salts also gave results similar to the analysis of the whole methylated oleoresin.

**Composition of fatty acids**

The composition of fatty acids (Table 5) in sapwood was different from that found in the heartwood. Fatty acids of sapwood contained more palmitic and stearic acids and contained lower amounts of the unsaturated fatty acids than did heartwood. With the transition from sapwood to heartwood, the proportion of unsaturated fatty acids was higher while the ratio of oleic acid to linoleic acid was lower. The heartwood fatty acids were similar to those present as esters in the heartwood (Tables 5 and 6). There was a marked difference in fatty acid composition between the fatty acids and the fatty acid esters of the sapwood. Palmitic acid comprised over 30 per cent of the fatty acids of sapwood but only six to ten per cent of the sapwood fatty acid esters were palmitates. The proportion of oleic acid in the fatty acids of sapwood was only about 30 per cent, but over 50 per cent of the fatty acid esters were oleates. The fatty acids of sapwood (Table 5) may be chemically unrelated to, and are perhaps physically separated from, the fatty acid esters of sapwood (Table 6). Perila and Manner (11) demonstrated that fatty acids were present within the middle lamella and fatty acids may be present in sapwood as remnants of cytoplasmic lipids. However, glyceride esters are believed to be present only in the ray parenchyma (12). The fatty acids in the heartwood (Table 5) are probably dominated by hydrolysis products of the fatty acid esters (Table 6) that are not metabolized in the sapwood.

In addition to those fatty acids listed in Table 5, there were significant amounts of other fatty acids present. From measurements of relative retention times, the branched chain C17 fatty acid was present in concentrations equal to or greater than the C16-1. There were also significant amounts of the C12, C14, and what appeared to be the $\Delta$ 5, 9, 12 C 18-3

**TABLE 3**

Composition of resin acids in *P. radiata* wood

| Sample | % of Total Resin Acid GLC Peak Area* | | | | | | |
|---|---|---|---|---|---|---|---|
| | Pim | San | Levo/Pal | Isop | Abie | Dehyd | Neo |
| **Tree 1** | | | | | | | |
| Outer sap | 6.8 | 1.5 | 54 | 1.2 | 7.5 | 8.2 | 22 |
| Inner sap | 8.5 | 1.8 | 51 | 1.3 | 7.5 | 7.0 | 24 |
| Outer heart | 5.9 | 1.2 | 47 | 1.2 | 15 | 4.5 | 24 |
| Inner heart | 7.3 | 1.0 | 50 | 1.1 | 13 | 4.5 | 23 |
| **Tree 2** | | | | | | | |
| Outer sap | 8.7 | 1.0 | 51 | 4.1 | 4.9 | 9.3 | 21 |
| Inner sap | 11 | 1.6 | 46 | 3.1 | 6.4 | 12 | 20 |
| Outer heart | 9.3 | 1.1 | 47 | 3.7 | 9.5 | 9.2 | 20 |
| Inner heart | 8.1 | 1.1 | 43 | 2.1 | 18.1 | 7.5 | 19 |
| **Tree 3** | | | | | | | |
| Sapwood | 8.4 | 1.1 | 53 | 2.7 | 4.5 | 10 | 20 |
| Heartwood | 8.5 | 1.3 | 44 | 3.6 | 12 | 14 | 17 |

* Pim = Pimaric acid, San = Sandaracopimaric acid, Levo/Pal = Levopimaric and Palustric acids, Isop = Isopimaric acid, Abie = Abietic acid, Dehyd = Dehydroabietic acid, Neo = Neoabietic acid.

**TABLE 4**

Composition of resin acids in *P. radiata* oleoresin

| Sample | Per cent of Total Resin Acid GLC Peak Area* | | | | | | |
|---|---|---|---|---|---|---|---|
| | Pim | San | Levo/Pal | Isop | Abie | Dehyd | Neo |
| Tree 4 | 0.42 | 1.6 | 63 | pnm† | 5.7 | 5.4 | 23 |
| Tree 5 | 0.24 | 1.5 | 57 | 5.4 | 5.5 | 4.8 | 25 |
| Tree 6 | 0.32 | 2.8 | 61 | pnm† | 6.2 | 2.7 | 26 |
| Ave | 0.33 | 2.0 | 60 | pnm† | 5.8 | 4.3 | 25 |

* For abbreviations see Table 3.
† pnm = present but not measurable.

**TABLE 5**

Composition of fatty acids in *P. radiata* wood

| Sample | Per cent of Total Fatty Acid GLC Peak Area | | | | | |
|---|---|---|---|---|---|---|
| | C16* | C18 | C18-1 | C18-2 | C18-3 | C20 |
| **Tree 1** | | | | | | |
| Outer sap | 35 | 16 | 38 | 10 | 2.0 | 1.0 |
| Inner sap | 31 | 16 | 37 | 15 | 2.2 | 1.2 |
| Outer heart | 14 | 5 | 41 | 28 | 10 | 1.5 |
| Inner heart | 17 | 11 | 35 | 24 | 11 | 1.2 |
| **Tree 2** | | | | | | |
| Outer sap | 30 | 7.1 | 33 | 22 | 1.8 | 4.7 |
| Inner sap | 28 | 7.3 | 36 | 25 | 1.6 | 2.5 |
| Outer heart | 10 | 2.6 | 49 | 35 | 1.1 | 2.0 |
| Inner heart | 9.6 | 2.5 | 47 | 36 | 1.1 | 3.8 |
| **Tree 3** | | | | | | |
| Sapwood | 34 | 8.6 | 32 | 22 | 1.3 | 2.2 |
| Heartwood | 17 | 4.8 | 44 | 30 | 1.6 | 2.6 |

* C16 = palmitic acid, C18 = stearic acid, C18-1 = oleic acid, C18-2 = linoleic acid, C18-3 = linolenic acid, C20 = arachidic acid.

**TABLE 6**

**Composition of fatty acid esters in *P. radiata* wood**

| Sample | Per cent of Total Fatty Acid Ester GLC Peak Area | | | | | |
|---|---|---|---|---|---|---|
| | C16* | C18 | C18-1 | C18-2 | C18-3 | C20 |
| **Tree 1** | | | | | | |
| Outer sap | 6.1 | 1.2 | 57 | 30 | 3.0 | 2.2 |
| Inner sap | 7.0 | 1.3 | 57 | 31 | 2.5 | 1.7 |
| Outer heart | 9.5 | 2.9 | 45 | 37 | 2.5 | 4.0 |
| Inner heart | 14 | 4.8 | 37 | 32 | 3.0 | 8.6 |
| **Tree 2** | | | | | | |
| Outer sap | 10 | 1.5 | 50 | 34 | 0.8 | 3.4 |
| Inner sap | 12 | 1.4 | 50 | 34 | 2.2 | 2.5 |
| Outer heart | 11 | 1.3 | 42 | 41 | 1.6 | 2.1 |
| Inner heart | 14 | 4.1 | 32 | 44 | 1.2 | 5.2 |
| **Tree 3** | | | | | | |
| Sapwood | 11 | 1.5 | 51 | 33 | 0.5 | 3.3 |
| Heartwood | 9 | 2.3 | 44 | 36 | 3.6 | 4.7 |

C16 = palmitic acid esters, C18 = stearic acid esters, C18-1 = oleic acid esters, C18-2 = linoleic acid esters, C18-3 = linolenic acid esters, C20 = arachidic acid esters.

fatty acids as were found by Hansen (5). However these components were not measured analytically nor were they completely identified.

**Composition of fatty acid esters**

The composition of the fatty acid esters did not differ between outer and inner sapwood (Table 6) but it changed significantly with the transition from sapwood to heartwood. Sapwood fatty acid esters contained small proportions of palmitate but were very rich in oleate esters. The proportion of oleate esters fell from 53 per cent in sapwood to about 40 per cent in the heartwood. This decrease in proportion of oleate esters was not accompanied by a proportional decrease in the amount of linoleate. In one inner heartwood sample the linoleate concentration of the fatty acid esters was greater than that of the oleate esters. The proportion of saturated fatty acid esters in the heartwood was higher than in sapwood. The low proportion of oleate compared with linoleate and saturated fatty acid esters in heartwood (Table 6) implied that metabolized esters (*i.e.* glyceride esters) were rich in oleate esters, while linoleate and saturated fatty acid esters were concentrated in other esters such as waxes. Assarsson and Akerlund (13) have demonstrated large differences in the fatty acid compositions of different lipid classes in *Pinus sylvestris* and *Picea abies* woods which lends support to this thesis.

In addition to these changes from outer sapwood to outer heartwood, there were also differences in the amounts (Table 2) and composition (Table 6) of fatty acid esters between outer and inner heartwood. Examination of the proportions and compositions of the total of fatty acids and fatty acid esters indicated that the low proportion of fatty acid esters within heartwood was not due to either hydrolysis to fatty acids or autoxidation. The major difference in composition — a lower proportion of oleate to linoleate esters — was evident in both the fatty acid esters and the fatty acids. Assuming no biological degradation of the fatty acid esters occurred within heartwood, it appeared that the more probable explanation was that the various wood samples originally contained different proportions of fatty acid esters that were not metabolized.

Further work, particularly in characterizing changes in the different classes of fatty acid esters, is needed to define the relationships between the fats and resins and heartwood formation.


## ACKNOWLEDGMENTS

The authors acknowledge the assistance of Mr J. Hare, Penola, South Australia, in obtaining wood samples and the expert analyses of Mr L. Lau. The authors also thank Mr R. V. Lawrence, Dr J. W. Rowe and Prof H. H. Bruun for gifts of resin acids.


## REFERENCES


(1) Buckland, N. J., Dalley, O. T., and Mathieson, C. S., *Appita* **7**, 165 (1953).
(2) McDonald, I. R. C., and Porter, L. J., *New Zealand J. Sci.* **12**, 352 (1969).
(3) Porter, L. J., *New Zealand J. Sci.* **12**, 687 (1969).
(4) Lawrence, R. V., Personal Communication, March 1970.
(5) Hansen, R. P., *New Zealand J. Sci.* **9**, 801 (1966).
(6) Nelson, P. J. and Hemingway, R. W., *Tappi.* In press.
(7) Zinkel, D. F., and Rowe, J. W., *Anal. Chem.* **36**, 1160 (1964).
(8) Hemingway, R. W., *Tappi* **52** (11), 2149 (1969).
(9) Mutton, D. B., In "Wood Extractives and their Significance to the Pulp and Paper Industries" (Edited by W. E. Hillis) p.331, Academic Press, N.Y. (1962).
(10) Mutton, D. B., *Tappi* **41**, 632 (1958).
(11) Perila, O., and Manner, P., *Paperi Ja Puu* **38**, 499 (1955).
(12) Back, E., *Svensk Papperstidn.* **63**, 793 (1960).
(13) Assarsson, A., and Akerlund, G., *Svensk Papperstidn.* **69**, 517 (1966).


Manuscript received for publication 15/9/70