

Oleoresins of Three Pinus Species from Malaysian Pine Plantations

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ABSTRACT

Turpentine and rosin of three Pinus species (Pinus caribaea var. hondurensis, P. merkusii and P. insularis) sampled at several plantations in Peninsular Malaysia were examined by co-chromatography with authentic samples on two columns of different polarity, capillary GCMS and Kovats retention index. Distillation of the oleoresins tapped from the pine trees yielded turpentine in the range of 25.0 to 38.8% (v/w). Turpentine derived from similar species at different sites showed little tree-to-tree variation in the chemical composition but with marked variation in the quantity of some components. All the oils were typical of turpentine constituents with α -pinene as the major constituent in the range between 65 and 94%. Other constituents which were common and abundantly found in all the species were β -pinene, camphene, myrcene, limonene and β -phellandrene. The resin acid composition of the rosin of similar species from different sites was very similar. The major components of the rosin were levopimaric / palustric acid which were in the range of 36.0 to 43.9% in P. var. hondurensis, 30.6 to 35.7% in P. merkusii and 35.2 to 45.1% in P. insularis. Other compounds identified were pimaric acid, isopimaric acid, dehydroabietic acid, abietic acid, neoabietic acid, sandaracopimaric acid and merkusic acid.

INTRODUCTION

Turpentine and rosin, produced by processing pine oleoresin, are used in a very wide variety of applications. The former is purchased by the chemical industry as a source of isolates for subsequent conversion to pine oil, fragrance and flavour compounds, pesticides, resin derivatives, solvent and thinner for paints and other technical products. Rosin is commonly used in the manufacture of soap, paints, adhesives, printing inks, coatings and paper-sizing (Greenhalgh, 1982). Pine oleoresin is obtained by tapping the living trunks of several Pine species. Amongst the species that have been exploited commercially for pine oleoresin are the temperate and sub-tropical group; *Pinus palustris*, *P. elliottii*, *P. caribaea*, *P. radiata* and *P. massoniana* and the tropical group; *P. roxburgii*, *P. kesiyana*, *P. merkusii*, *P. oocarpa* and *P. caribaea var. hondurensis* (Copper et al., 1984).

Three *Pinus* species (*Pinus caribaea var. hondurensis*, *P. merkusii* and *P. insularis*) were established in Peninsular Malaysia in the early 1950's as reforestation crop (Vincent et al., 1965). Encouraging results from the trials led to the plantation of the three *Pinus* species on a pilot scale in 1967 for an eventual production of raw material for pulping (Freezaillah, 1967). Pilot plantations of the exotic coniferous species, mainly *P. caribaea var. hondurensis* were expanded to Sabah, East Malaysia (Hong, 1981). Tapping trials were carried out on *P. caribaea var. hondurensis*, grown in an experimental plot at the Forest Research Institute Malaysia (Low and Abdul Razak, 1985). However, the potential of the pine trees as potential source of gum naval stores (turpentine and rosin) has not been exploited.

This paper reports on the quantitative and qualitative chemical analyses of turpentine and rosin of three *Pinus* species viz., *P. caribaea* var. *hondurensis*, *p. merkusii* and *p. insularis*, sampled at several pine plantations in Peninsular Malaysia. This study is the first step in evaluating the standing resource of Malaysian pine plantations as potential source for the production of naval stores.

MATERIAL AND METHODS

Samples of oleoresins were collected from the *Pinus* species at several plantation sites in Peninsular Malaysia (Table 1). Within each site seven trees of each species were tapped for their oleoresins. Tapping was carried out by drilling a slight sloping hole into the wood at breast height and a tightly fitting glass vial was inserted into the hole. The oleoresin accumulated in the vial was collected on the next day and stored in the refrigerator. The oleoresin samples (20g) were water-distilled for 8 h in an essential oil extraction apparatus to yield turpentine and rosin as the volatile and non-volatile fractions respectively. Mean yield of turpentine from the oleoresin (%v/w) of each species from within each site were calculated. Turpentine isolated was dried over anhydrous sodium sulfate.

The essential oil was analysed by gas chromatography. Shimadzu model GC 14A chromatograph equipped with FID detector was used with fused-silica capillary columns of different polarity (SE 30 and PEG 20; 25m x 0.25 mm i.d.). The operating parameters were: nitrogen as carrier gas at 50 cm³ min⁻¹, injector temperature 230 deg C, detector temperature 230 deg C. The non-polar column was programmed initially at 60 deg for 10 min, then 30 deg C min⁻¹ to 180 deg C for 1 min. The polar column was programmed at the following conditions; initial temperature 50 to 230 deg C at 3 deg C min⁻¹. The oils were also analysed by GCMS with Hewlett-Packard GCMSD 5890 series 2 mass spectrometer (70 eV direct inlet) on an SE 30 capillary column (25 m x 0.2 mm i.d.) initially at 60 deg C for 10 min, then programmed at 3 deg C min⁻¹ to 180 deg C with helium as carrier gas. The constituents were identified by co-chromatography on the different columns with authentic samples and by comparison of their Kovats indices with literature values and their mass spectral data with those from the Wiley mass spectral database. Kovats indices were obtained from the gas chromatogram by logarithmic inter-polation between bracketing alkanes. The homologous series of n-alkanes were used as standards (Kovats, 1965).

Resin acids in rosin were determined initially by converting the acids to the more volatile methyl esters followed by GC and GCMS analyses. Methylation was carried out by initially converting the acids to tetramethylammonium salts as follows; rosin sample (10 mg) was dissolved in dry methanol (1.0 ml), followed by 1 drop of phenolphthalein and 2 drops of 20% tetramethylammonium hydroxide in methanol. The solution turning purple indicated the formation of salts. A portion of the prepared sample was injected onto the chromatographic column and the tetra-methylammonium salts were pyrolysed to methyl esters in the injection port of the chromatograph. The prepared esters were carried through a

fused silica capillary column SE 30 (25m x 0.2 i.d.) with nitrogen as the carrier gas. The column was programmed at an isothermal condition of 200 deg C. Individual acids were identified by a data and by comparing their mass spectral data with those from the Wiley mass spectral database.

Acid number of rosin was determined by titrating a sample of the rosin (2.0g) in 95% ethanol with standard alkali solution (0.5N KOH) manually using phenolphthalein as indicator.

Table 1 - Pine plantation sites in Peninsular Malaysia

Site	Year planted	Mean diameter (cm)
Selangor:		
1. FRIM, Kepong		
P caribaea	1961	39.5
P.merkusii	1961	48.2
P.insularis	1961	40.3
2. Batu Arang		
P caribaea	1956	43.9
P.merkusii	1963	39.4
P.insularis	1974	36.3
3. Sungai Buloh		
P caribaea	1957	20.6
P.merkusii	1955	29.9
P.insularis	1957	41.2
4. Jalan Ipoh		
P.merkusii	1961	30.6
Kedah:		
Kulim		
P caribaea	1962	43.8
P.merkusii	1963	33.9
P.insularis	1962	33.5
Perlis:		
Mata Air		
P caribaea	1960	44.8
Johore:		
Ulu Sedili		
P caribaea	1975	27.2
P.merkusii	1973	38.4
Pahang:		
Kemasul		
P caribaea	1974	30.6

RESULTS AND DISCUSSION

Mean yields of turpentine obtained from water distillation of the *Pinus* species from different sites were in the following ranges: *P. caribaea* var *hondurensis* (25.0-34.6 %); *P. merkusii* (27.8-36.4 %); *p. insularis* (28.6-38.8 %). Collection of the oleoresins in glass vials inserted in the tree stem ensured that loss of turpentine due to evaporation was minimal.

Mean percentages for each constituent of the turpentine derived from the *Pinus* species within each site are shown in Table 2-5. The data show that there were little variations in turpentine composition of oleoresins within species from different sites but the concentration of some components showed major variations. *P. caribaea* var. *hondurensis* turpentine of five different sites (FRIM, Sungai Buloh, Kemasul, Batu Arang and Kulim) showed very little variations in composition and proportion of each constituent with α -pinene as the major constituent (72.7-79.4 %) and the presence of appreciable amounts of β -pinene (3.1-6.4 %), limonene (1.5-5.5%) and methyl chavicol (1.0-3.7%) (Table 2). However, turpentine from two other sites (Ulu Sedili and Mata Air) contained lower concentrations of α -pinene (65.6-68.4%) but higher concentrations of limonene (18.9-20.5%). The presence of α -himachalene and relatively large number of other associated sesquiterpenes (high boilers), although in small amounts and the absence of 3-carene, could further distinguish this species from the others.

There was marked variation in the α -pinene content of turpentine of *Pinus merkusii* between trees from six different sites (Table 3). Turpentine derived from trees at FRIM, Ulu Sedili, Kulim and Batu 8 Japan Ipoh contained α -pinene in the range of 75.7-88.6% while trees at 2 and 4 contained lesser amount of the compound (53.9-56.1%) but higher concentrations of β -phellandrene (10.0-10.8%) and limonene (14.3-25.2 %).

Turpentine from this species is distinguished from the other species in containing negligible amount of sesquiterpenes and being entirely absent from some trees. The presence of 3-carene in appreciable amounts (0.2-7.9%) further distinguished this species from the others.

Pinus insularis turpentine derived from trees at different sites exhibited very little tree-to-tree variation in composition and proportion of each constituent (Table 4). They were characterised in containing the highest concentration of α -pinene (87.8-94.3%) among the species studied. Other compounds which were present at more than 1% concentration were camphene, β -phellandrene, limonene and β -pinene.

Table 2 - Chemical constituents of turpentine derived from *Pinus caribaea* var *hondurensis* (column; 25 m SE 30)

Compound	Kovats index	1	2	3	4	5	6	7	Identification
Tricylane	923	0.1	tr	0.1	0.2	0.2	0.2	0.2	MS, KI
α - pinene	936	75.5	79.4	72.7	78.1	65.6	77.1	68.4	MS, KI, Co
Camphene	948	1.3	1.5	1.2	1.4	1.2	1.6	1.2	MS, KI, Co
Sabinene	962	0.1	tr	0.1	0.2	0.3	tr	0.2	MS, KI, Co
α - pinene	974	3.6	5.8	5.5	tr	4.1	6.4	5.5	MS, KI, Co
Myrcene	983	1.5	1.5	1.6	2.0	1.6	1.4	2.0	MS, KI, Co
α - Phellandrene	998	0.3	0.3	0.6	0.4	1.2	0.4	0.8	MS, KI, Co
3-Carene	1003	tr	tr	-	-	tr	-	tr	MS, KI, Co
α - Terpinene	1013	0.1	0.1	-	-	-	-	0.1	MS, KI, Co
p-Cymene	1015	-	tr	-	-	tr	tr	-	MS, KI, Co
δ -Phellandrene	1026	-	6.2	3.6	-	5.0	1.6	1.0	MS, KI, Co
Limonene	1026	5.5	1.5	1.7	4.3	20.5	3.5	18.9	MS, KI, Co
(Z)- β - Ocimene	1028	tr	-	-	1.4	tr	tr	-	MS, KI, Co
γ - Terpinene	1057	tr	tr	tr	tr	tr	tr	0.1	MS, KI, Co
Terpinolene	1081	tr	0.2	0.1	0.1	0.1	0.2	0.2	MS, KI, Co
allo - Ocimene	1083	tr	tr	tr	tr	tr	tr	tr	MS, KI, Co
Linalol	1085	0.1	tr	-	0.1	-	-	0.1	MS, KI, Co
Methyl chavicol	1179	3.6	1.0	3.7	0.8	4.3	1.2	1.2	MS, KI
Longipinene	1354	105	0.6	1.9	0.7	0.5	0.2	0.2	MS, KI
Methyl eugenol	1372	tr	tr	tr	tr	tr	tr	0.1	MS, KI
Longifolene	1400	0.1	tr	0.1	0.2	0.1	tr	0.2	MS, KI
β - Caryophyllene	1405	tr	tr	tr	tr	tr	tr	tr	MS, KI, Co
Sativene	1421	-	tr	tr	-	tr	tr	-	MS, KI
(z)- β -farnesene	1446	tr	tr	tr	tr	-	-	-	MS, KI, Co
β - Bimabolene	1503	tr	-	tr	tr	tr	tr	-	MS, KI
β - Himachalene	1517	tr	tr	-	-	tr	-	-	MS, KI

1= FRIM; 2 = Sungai Buloh; 3= Kemasul; 4= Batu Arang; 5= Ulu Sedili; 6= Kulim; 7= Mata Air; 8 = Batu 8 Jalan Ipoh; *Percentages were means of seven trees asid calculated based on the concentration obtained on columns SE 30; the Kovats index of compounds on the PEG 2aH column was also determined: MS = mass fragmentation; KI = retention index; Co = co-chromatography with authentic sample.

Table- 3 - Chemical composition of Turpentine derived from *Pinus merkusii*

Compound	Percentage					
	1	2	4	5	6	8
Tricyclene	0.2	0.1	0.3	0.4	tr	0.1
α -Pinene	82.6	53.9	56.1	75.7	88.6	86.4
Camphene	0.8	0.6	0.6	0.7	0.8	0.8
Sabinene	tr	tr	0.4	0.3	1.0	0.1
β -Pinene	1.3	1.1	0.8	5.3	5.5	0.9
Myrcene	0.8	2.9	2.6	1.5	0.9	0.9
α -Phellandrene	5.5	-	-	8.0	7.8	-
3-Carene	5.5	0.5	10.3	0.2	1.0	7.9
α -Terpinene	tr	tr	0.2	0.3	tr	0.1
p-Cymene	tr	tr	tr	tr	tr	tr
β -Phellandrene	0.9	10.8	1.0	0.3	0.2	0.2
Limonene	0.6	25.2	14.3	1.0	0.5	0.4
γ -Terpinene	tr	0.4	0.3	0.3	tr	0.1
Terpinolene	0.8	0.1	3.0	2.9	0.8	0.7
Longipinene	1.0	-	-	-	-	1.0
Longifolene	0.4	-	-	-	-	0.3

See Table 2 for legends

Table- 4 - Chemical constituents of turpentine derived from *Pinus insularis*

Compound	Percentage			
	1	2	4	6
Tricyclene	0.3	0.3	0.4	0.3
α -Pinene	92.2	94.3	87.8	92.6
Camphene	1.0	1.0	4.5	2.1
Sabinene	0.1	tr	0.2	tr
β -Pinene	1.0	1.0	1.1	0.9
Myrcene	0.7	0.6	0.7	0.7
α -Phellandrene	0.3	0.2	0.5	tr
3-Carene	0.1	tr	0.2	0.2
α -Terpinene	tr	tr	tr	tr
p-Cymene	tr	1.5	-	-
β -Phellandrene	4.5	1.2	3.9	1.1
Limonene	0.7	tr	2.0	1.5
γ -Terpinene	0.2	0.3	tr	0.2
Terpinolene	0.5	-	0.9	0.7
Longipinene	0.2	-	tr	0.1
β - Caryophyllene	0.3	-	tr	0.1
(Z) - β - Farnesene	0.3	-	0.1	tr

See Table 2 for legends

The resin acid composition and acid number of rosins of *Pinus* species for each provenance are shown in Tables 5-7. Examination of the data reveals that there were similarities in the resin acid composition of rosins of similar species from different sites. The quantitative differences between them were less significant. Levopimaric/palustric acids were the most abundant components in all the rosin samples with the following ranges in each species; *P. caribaea* var. *hondurensis* (36.0-43.9 %); *P. merkusii* (30.6-35.7 %); *p. insularis* (35.2-45.1 %). The resin acid composition of *p. caribaea* var *hondurensis* rosin closely resembled those of *p. insularis* as they exhibited compositional and quantitative similarities (Table 4&6). Other than levopimaric/palustric acids, abietic (14.6-19.5%) and neoabietic acids (14.2-22.3%) were also present in significant quantities in both rosins. Values of acid number for rosins of both species were also similar, i.e. in the range of 154.9-160.5.

Table 5 - Rain acid composition (%)* and acid number of *Pinus hondurensis* rosin

Compound	Percentage @								
	1	2	3	4	5	6	7	8	RT%
Pimaric	5.9	5.0	4.1	4.5	4.7	4.7	4.1	4.4	17.6
Sandaracopimaric	1.9	1.5	1.8	1.7	1.7	1.3	1.8	1.6	18.5
Isopimaric	8.1	12.1	10.2	10.8	9.4	11.2	11.3	11.6	20.9
Levopimaric / palustric	43.9	39.4	42.0	41.1	39.9	36.0	36.0	41.7	21.6
Dehydroabietic	9.7	6.5	5.0	8.4	8.5	8.0	7.9	6.1	23.1
Abietic	14.6	17.1	14.7	16.3	17.8	19.5	19.2	17.4	27.1
Neoabietic	15.6	16.0	22.3	16.2	15.4	19.0	19.2	16.8	31.6
Merkusic	-	-	-	-	-	-	-	-	34.4
Acid number	155. 2	158. 1	155. 9	156. 7	160. 5	157. 9	155. 5	157. 0	

* Analysed as methyl esters, @ Mean of seven trees and calculated based on the concentration obtained on column SE 30; RT # = Retention time of the methyl esters on column SE 30 at isothermal condition (200 deg C). See Table 2 for other legends.

Rosin from *p. merkusii* was different from the other *Pinus* species in that it contains merkusic acid (5.9-7.2%) and appreciable amounts of sandara-copimaric acid (6.4-11.4%), and the absence of pimaric acid. Isopimaric acid present at relatively large amounts (19.2-24.4%), could further distinguish *P. merkusii* from the other species. The acid number for the rosin was relatively high, ranging from 170.5 to 183.0, due to the presence of merkusic acid which is a dicarboxylic acid (in contrast to the other resin acids which are monocarboxylic) (Table 6).

Table 6. - Resin acid composition (%)* and acid number of resin derived from *Pinus merkusii*

Compound	Percentage @					
	1	2	4	5	6	8
Pimaric	-	-	-	-	-	-
Sandaracopimaric	10.0	9.0	6.4	8.1	9.9	11.4
Isopimaric	24.4	23.7	22.6	22.7	19.2	20.3
Levopimaric / palustric	31.2	30.6	31.6	33.6	32.8	35.7
Dehydroabietic	6.4	2.2	3.4	3.5	6.0	3.6
Abietic	14.4	17.3	19.6	20.4	17.1	16.0
Neoabietic	5.4	9.2	8.8	8.0	7.6	5.4
Merkusic	7.2	6.3	6.8	6.1	5.9	6.9
Acid number	175. 2	177. 8	179. 5	170. 5	170. 3	176. 4

See Table 2 & 5 for legends

Table 7 - Resin acid composition (%)* and acid number of toxin derived from *Pinus insularis*

Compound	Percentage @			
	1	2	4	6
Pimaric	5.7	8.6	2.2	8.6
Sandaracopimaric	1.4	1.7	1.0	2.2
Isopimaric	12.8	7.1	7.8	7.5
Levopimaric/ Palustric	35.2	39.7	45.1	40.8
Dahydroabietic	4.6	4.6	4.3	4.6
Abietic	17.3	19.2	16.0	17.8
Neoabietic	18.3	14.4	20.0	14.2
Nerkusic	-	-	-	-
Acid number	154.9	155.2	155.0	156.6

See Table 2 & 5 for legends

The chemical compositions of turpentine and rosin of the three *Pinus* species of Malaysian pine plantations were similar to the composition characteristics of typical commercial turpentine and rosin (Hall, 1979). The similarities and differences in turpentine and resin acid compositions could be useful in species characterization, especially in distinguishing between *Pinus* species which are morphologically similar.

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