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Chemical Composition of Indonesian *Pinus merkusii* Turpentine Oils, Gum Oleoresins and Rosins from Sumatra and Java

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Abstract: Turpentine oils, gum oleoresins and rosins from *Pinus merkusii* Jungh et de Vrises were collected on the island of Sumatra and Java for investigation of their chemical composition. Gum oleoresins were separated into neutral and acidic fractions with an aqueous 4% sodium hydroxide solution. The fractions containing turpentine oils and rosins were analyzed by Gas Chromatography (GC) and Gas Chromatograph Mass Spectrometry (GC-MS), respectively. The neutral fractions of gum oleoresins and turpentine oils mainly consisted of α-pinene, Δ-3-carene and β-pinene. A difference in chemical composition with locality was recognized for the turpentine oils and neutral fractions of gum oleoresins. While examining the chemical composition of the acidic fractions of gum oleoresins and rosins, we found that relative retention time could not be used to identify each resin acid. Based on mass spectral comparison, the major constituents of the acidic fractions and rosins were identified as sandaracopimaric acid, isopimaric acid, palustric acid, dehydroabietic acid, abietic acid, neoabietic acid and merkusic acid. The major component of the acidic fractions was palustric acid, while that of both rosin 1 and 2 was abietic acid. The chemical composition of the acidic fractions of gum oleoresins and rosins differed with locality. Using TC-1 and TC-5 columns, levopimaric acid could not be separated from rosins or acidic fractions of gum oleoresins of Indonesian *Pinus merkusii*. This is the first report that neither fraction contains levopimaric acid.

Key words: *Pinus merkusii*, turpentine oil, pine gum oleoresin, rosin, neutral fraction, acidic fraction

INTRODUCTION

Pinus merkusii is an important plant used on plantations in Indonesia. Its wood is used as raw materials in the work-working and pulp-paper industries and also produces a gum resin containing a high quality rosin and turpentine oil[1]. Pine forests in Indonesia cover around 300,000 ha and the production capacity of pine gum resin is more than 500,000 tons a year. This species grows naturally in Aceh, North Sumatra and Jambi; while pine plantations cover West Sumatra, Java, South Sulawesi, North Sulawesi and South-East Sulawesi. Indonesia is the third largest rosin-producing country in the world after China and Portugal[2]. Among Non-wood Forest Products (NWFPs), rosin is the second biggest produce in Indonesia after rattan, being used in perfume, as a flavor in industry and for various other purposes. In the last ten years, it is estimated that Indonesia has been producings about 40,000 tons of rosin a year and about 10,000 tons of turpentine products a year[3].

There are many Rosin and Turpentine industries in Indonesia, mostly on Java and in North Sumatra. These industries obtain pine gum resin from large areas of pine forests. The industry in North Sumatra obtains pine gum resin tapped in West Sumatra as well as in North Sumatra itself. The Rosin and Turpentine industries in West Java attain their raw materials from pine forests in the West Java and Banten provinces. Hence, the Rosin and Turpentine industries in Central Java and East Java also obtain raw materials from their own province. Research on the chemical composition of the products of these industries is very rare[4,5] and this may not truly represent the chemical constituents of pine gum resin, turpentine oil and rosin from Indonesia as the samples only came from pine stands in certain parts of Jambi and North Sumatra. To identify the chemical composition of these products more precisely, pine gum resin or rosin samples should be obtained from wide range of areas. This can be accomplished by taking samples directly from the Rosin and Turpentine companies. The objective of this study was to characterize and identify the chemical composition of turpentine oil, pine gum and rosin from several companies.

MATERIALS AND METHODS

This research project was conducted in the Faculty of Agriculture, Ehime University, Japan and the Forest

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Fig. 1: Locations where samples were collected

Product Technology Research and Development Center, in Indonesia during 2004 in an effort to clarify the chemical composition of turpentine oils, gum oleoresins and rosin from *Pinus merkusii* for the preparation of fortified rosins.

Sample origin: Rosins (R1-R3), turpentine oils (TP1-TP3) and pine gum oleoresins (GO1-GO4) of *Pinus merkusii* were used as samples in this experiment. Both the rosins and turpentine oils were collected from Processing companies in East Java, West Java and North Sumatra in Indonesia in 2004. Further, pine gum oleoresin (GO4) was also collected from West Sumatra (Fig. 1). Rosins and turpentine oils were produced by the companies through steam distillation.

Separation of gum oleoresins into acidic and neutral fractions: Approximately 1-2 g of each sample of the gum oleoresins was dissolved in diethyl ether, transferred to a 50 mL separation funnel and then added to an aqueous 4% sodium hydroxide (NaOH) solution. After shaking, the soluble fraction was removed and then the aqueous 4% NaOH solution was added to the funnel again. The same treatment was repeated. To obtain the soluble fraction, an aqueous 16 N Hydrochloric acid (HCl) solution was added to make an acidic fraction and then extracted twice with diethyl ether. The ethereal solution was dried over anhydrous sodium sulfate overnight. Each fraction was obtained after evaporation of the solvent with an evaporator *in vacuo*. Each insoluble ethereal fraction was dried over anhydrous sodium sulfate overnight to obtain a neutral fraction after evaporation of the solvent with the evaporator *in vacuo*. The acidic fraction was methylated with a diazomethane ethereal solution using standard procedures. Both the acidic and neutral fractions were dissolved with chloroform and stored in a freezer prior to analysis.

Analysis of neutral fractions of gum oleoresins and turpentine oils: The GC analyses of neutral fractions of gum oleoresins (NF1-NF4) and turpentine oils (TP1-TP3) were performed using a HITACHI 3000 Gas chromatograph, equipped with an electronic Chromato-integrator D-2500, a FID detector, an injector and a TC-1 capillary column (30 m× 0.25 mm i.d. and film thickness 0.25 μm). The column temperature was 70°C, rising at 2°C min^{-1} to 200°C. The carrier gas was Helium delivered at a flow rate of 1.70 mL min^{-1} with a split ratio of 1:10. The FID detector and injector port were maintained at a temperature of 230 and 230°C, respectively.

Analysis of acidic fractions of gum oleoresins and rosins: The GC analyses of acidic fractions of gum oleoresins (AF1-AF4) and rosins (R1-R3) were performed using the same Gas chromatograph. The column temperature was 150°C, rising at 4°C min^{-1} to 300°C. The carrier gas was Helium delivered at a flow rate of 1.70 mL min^{-1}. The FID detector and injector port were maintained at a temperature of 260 and 300°C,

respectively. Acid fractions of gum oleoresins and Rosins were also analyzed using a TC-5 capillary column (30 m×0.25 mm i.d. and film thickness 0.25 μm), with the FID detector and injector port maintained at 280 and 280°C, respectively. The column temperature was 170°C, rising at 2.5°C min^{-1} to 280°C. The carrier gas was Helium delivered at a flow rate of 2.32 mL min^{-1} with a split ratio of 1:20. Using the same column and conditions, both acids were also analyzed isothermally at 200°C.

Identification and quantification of constituents: The main constituents of the neutral fractions and turpentine oils were identified by comparing retention times with those of authentic samples. Results were confirmed based on relative retention indices (KI) determined by injecting a reference mixture of C_8 to C_{16} hydrocarbons into the GC system under the same conditions as for the analysis and calculated according to a formula in the literature[6-8].

The main constituents of the rosins and acidic fractions were identified using a Shimadzu QP 5050A Gas chromatograph Mass spectrometer. Two capillary columns (TC-1 and TC-5, 30 m×0.25 mm i.d., film thickness 0.25 μm) were used for the identification. The analysis was performed using the same conditions as in the GC analysis. Many compounds were identified using Wiley libraries (7th Edn.) by comparing mass spectra for the injected samples to mass spectra in the library. Some papers were also helpful for the identification of compounds[5,9,10].

The quantification of constituents was conducted with a GC-FID profile obtained on a capillary column according to the peak area percent method without response factor correction[11].

RESULTS AND DISCUSSION

Analysis of neutral fractions of gum oleoresins and turpentine oils: Pale yellow neutral fractions obtained by the separation of gum oleoresins with aq 4% NaOH, which has a similar odor to pine, amounted to 13-23%. Authentic samples were used as references to identify the constituents of neutral fractions and turpentine oils. Sabinene was identified by comparing its retention index to published data[6,12]. An example of the GC profile for neutral fractions and turpentine oils is shown in Fig. 2.

Turpentine oils contained around 82-86% α-pinene and 8-12% Δ-3-carene (Table 1). This result is consistent with limited published data; however, the α-pinene content of turpentine oils was higher than previously reported, except for turpentine oil from Lobogala[4]. These results could be attributed to how the research samples were obtained. As explained before these samples were taken from the Rosin and Turpentine Companies, which obtain pine gum oleoresin from a wide range of pine forests of various ages and in different climates; while the sample for the previous study came from several merkus pine stands. Moreover, neutral fractions contained 57-75% α-pinene and 15-26% Δ-3-carene. Limited published data indicated that the sabinene in turpentine oil from Thai and Filipino merkus pine could be separated from β-pinene; while myrcene could be separated from Δ-3-carene[13], but this was not the case for turpentine oil from Indonesian merkus pine[4]. In addition, using a TC-1 in this experiment, sabinene and capillary column myrcene could also be separated from β-pinene and from Δ-3-carene, respectively.

Table 1: Composition of neutral fractions and turpentine oils

	Percentage							
Constituents	NF1	NF2	NF3	NF4	TPT1	TPT2	TPT3	RI
α-pinene	57.7	73.1	74.3	71.8	86.4	82.9	82.4	945
d-camphene	1.0	0.8	0.7	0.9	0.9	0.9	0.8	954
Sabinene	0.1	0.0	0.0	-	-	-	-	973
β-pinene	4.8	1.8	0.8	0.1	2.2	2.2	2.4	981
Myrcene	1.0	0.7	0.7	8.8	0.3	0.4	0.6	993
α-phellandrene	0.0	0.2	0.1	0.1	0.1	0.4	0.1	1003
Δ-3-carene	26.0	16.0	22.0	15.0	8.8	11.0	12.0	1016
p-cymene	0.6	0.8	0.0	0.4	0.2	1.1	0.3	1021
d-limonene	2.8	1.9	0.9	1.7	0.9	1.3	1.4	1028
α-terpineol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1174
β-caryophyllene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1411

NF1 = Neutral fraction from East Java; TPT1 = Turpentine oil from East Java;
NF2 = Neutral fraction from West Java; TPT2 = Turpentine oil from West Java;
NF3 = Neutral fraction from North Sumatra; TPT3 = Turpentine oil from North Sumatra;
NF4 = Neutral fraction from East Java;
RI = Retention indices in elution on the TC-1 column (equal to DB1 and OV1 column)

Table 2: Relative retention time of compounds from acidic fractions and rosins eluted on two columns (TC-1 and TC-5)

	TC-1	TC-5	TC-5 isothermal	Ref[9]	Ref[5]	Ref[10]
Constituents	RRT					
Pimaric acid ME	-	-	-	0.88	0.88	0.90
Sandaracopimaric acid ME	0.93	0.9	0.77	0.91	0.90	0.92
Isopimaric acid ME	0.97	0.95	0.88	0.95	0.95	0.95
Palustric acid ME	0.98	0.96	0.91	0.97	0.97	0.96
Dehydroabietic acid ME	1.00	1.00	1.00	1.00	1.00	1.00
Abietic acid ME	1.05	1.05	1.15	1.05	1.05	1.05
Neoabietic acid ME	1.10	1.12	1.34	1.11	1.12	1.12
Merkusic acid ME	1.12	1.14	1.46	-	0.00	1.22

RT dehydroabietic acid ME on TC-1; 20.790 min; RT dehydroabietic acid ME at 26.821 min on TC-5;
RT dehydroabietic acid ME isothermally eluted on TC-5 at 35.123 min;
RTT= Relative Retention Time
Reference 1= no RT for dehydroabietic acid ME; Reference 2= RT of dehydroabietic acid ME 26.562 min;
Reference 3= RT of dehydroabietic acid ME 28.305 min

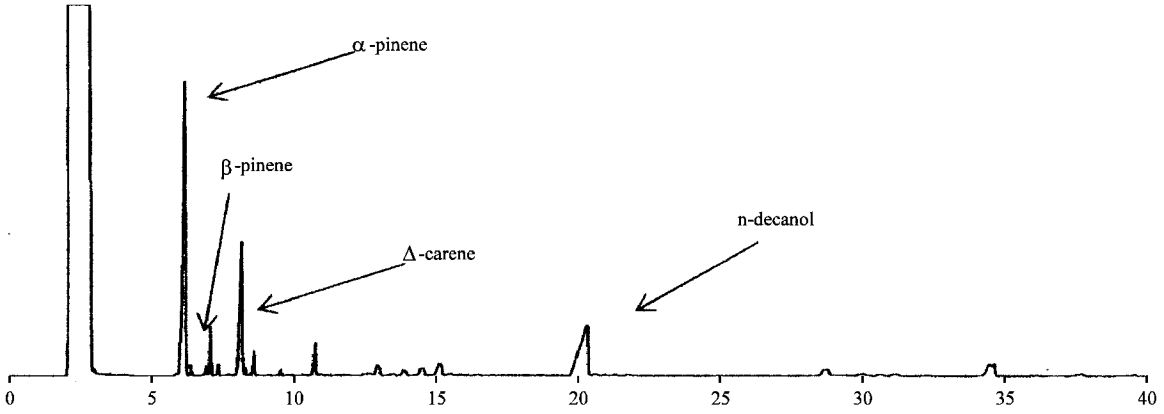

Fig. 2: An example of a GC-FID profile of the neutral fraction and turpentine oil on a TC-1 column

As mentioned above, all neutral fractions and turpentine oils mainly contained α-pinene followed by Δ-3-carene. However, there was a significant difference in content, with the amount of α-pinene greater in turpentine oils than in neutral fractions. On the other hand, levels of Δ-3-carene were lower. Moreover, based on the locations of merkus pine stands, the difference among the sites appears to follow a pattern according to the location where the samples were taken, with the proportion of α-pinene increasing in neutral fractions, but decreasing in turpentine oils from east to west (Table 1 and Fig. 2). This could be attributed to seasonal variation, with West Java tending to be more humid. The further west the site, the more rain it receives. The variation in the quantitative composition of monoterpenes, such as in turpentine oil, also depended to a large extent on genetic factors[12]. Another possibility is attack from herbivores as happened in a population of ponderosa pine causing a decrease in α-pinene[14]. These factors would all influence the quality of pine gum oleoresin as a source of raw material for the distillation process in the Rosin and Turpentine Industries.

Analysis of acidic fractions of gum oleoresins and rosins: After separation of gum oleoresins with the aq 4% NaOH solution, yellowish acidic fractions (AF1-AF4), which had a similar odor to pine, were obtained in relatively good yields, 66-79%. Identification of the main constituent of the acidic fractions (AF1-AF4) and rosins (R1-R3) using relative retention times was somewhat difficult. From the calculation of the relative retention times of AF1 to AF4 and R1 to R3, the relative retention times drawn from both columns (TC-1 and TC-5) gave different values. Hence, the relative retention times from the literature were also different[5,9,10] (Table 2). This demonstrates that the chemical constituents of acidic fractions and rosins from Indonesian *Pinus merkusii* could not be identified using relative retention times. The constituents were then identified by comparing the mass spectrum of each peak with that in the libraries.

The results of chemical identification for acidic fractions and rosins using mass spectra indicated that there are seven major constituents; sandaracopimaric acid methyl ester (ME), isopimaric acid ME, palustric ME, dehydroabietic acid ME, abietic acid ME, neoabietic

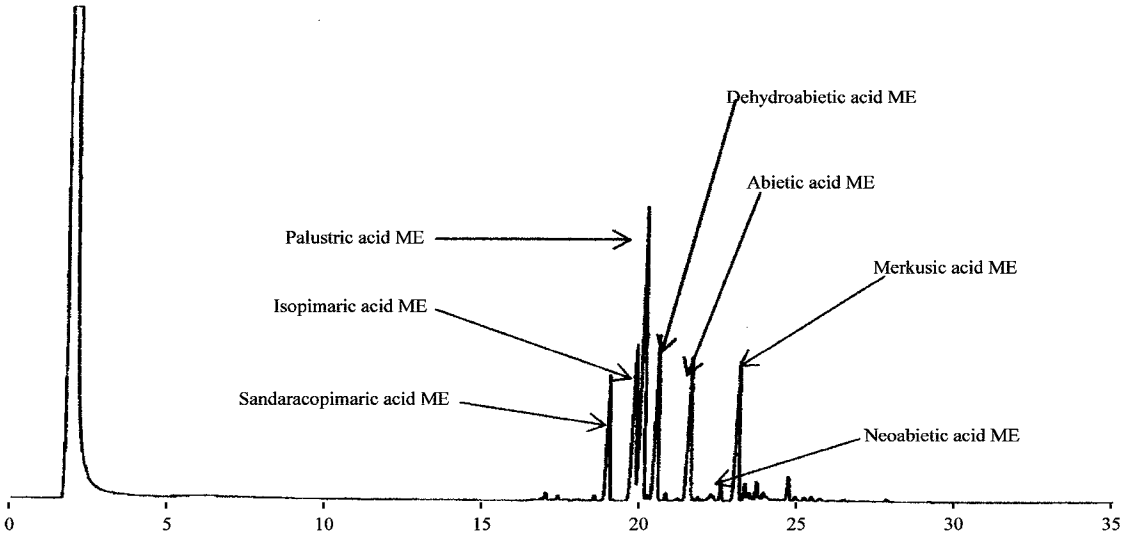

Fig. 3: An example of a GC-FID profile of the acidic fraction and rosin on a TC-1 column

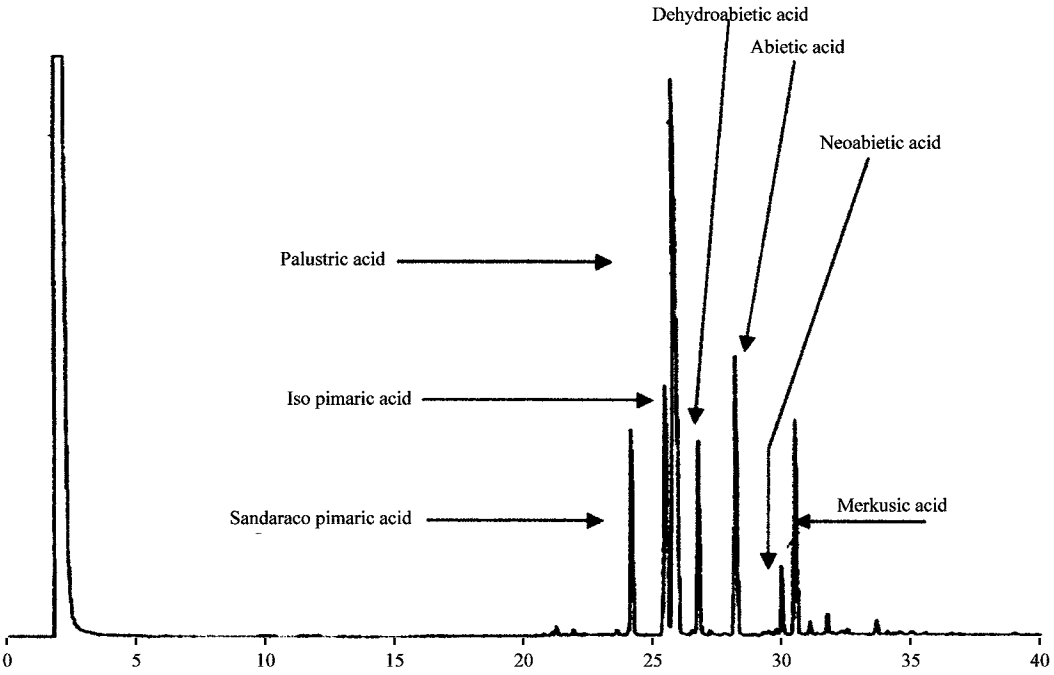

Fig. 4: An example of GC-FID profile of the acidic fraction and rosin on a TC-5 column (Gradient temperature)

acid ME and merkusic acid ME. Examples of GC-FID profiles for acidic fractions and rosins on TC-1 and TC-5 (both gradient and isothermal temperature programmes) columns (Fig. 3-5). The GC-FID profiles using both columns are similar, including the TC-5 column with the isothermal temperature programme. However, the TC-1 column gave a shorter retention time. Using the TC-5 column, isothermal temperature programme gave a longer retention time than did the gradient temperature programme; however, even though this method took more time, each constituent of the samples could be separated perfectly. Further, these major constituents of acidic fractions and rosins of Indonesian *Pinus merkusii* were quantitatively similar to the corresponding data in the literature[4,5,9,13]. However, the data from the previous studies showed that palustric acid ME and levopimaric acid ME always eluted together and were calculated as a single value. In the present experiment, even though rosin

Table 3: Composition of Acidic fractions eluted on TC-1 and TC-5 columns

	Acidic fraction 1 (%)			Acidic fraction 2 (%)			Acidic fraction 3 (%)			Acidic fraction 4 (%)		
Constituent	TC-1	TC-5a	TC-5b	TC-1	TC-5a	TC-5b	TC-1	TC-5a	TC-5b	TC-1	TC-5a	TC-5b
Pimaric acid ME	-	-		-	-		-	-		-	-	
Sandaracopimaric acid ME	9.8	11.2	9.3	17.1	7.4	6.9	9.2	9.9	9.3	10.2	9.65	11.6
Isopimaric acid ME	16.5	17.2	14.2	17.1	19.9	20.0	20.3	20.4	19.9	7.3	14.6	16.5
Palustric acid ME	31.8	33.4	29.0	32.2	38.2	33.9	36.5	37.6	33.2	42.7	40.3	35.3
Dehydroabietic acid ME	15.3	14.4	14.1	6.7	7.1	9.3	4.5	4.0	7.0	9.4	8.18	11.5
Abietic acid ME	13.2	12.3	17.5	13.2	14.1	18.0	13.5	13.3	14.6	15.7	14.3	14.6
Neoabietic acid ME	1.2	1.3	1.3	3.2	3.3	3.6	4.2	4.2	4.5	3.0	2.85	3.32
Merkusic acid ME	12.2	10.2	14.6	10.6	9.9	8.3	11.9	10.6	11.7	11.7	10.1	7.11
	100.0	100.00	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.00	100.00

TC-1 and TC-5a: Gradient temperature programme, TC-5b: Isothermal temperature programme, Acidic fraction 1: Sample from East Java, Acidic fraction 2: Sample from West Java, Acidic fraction 3: Sample from North Sumatra, Acidic fraction 4: Sample from West Sumatra

Table 4: Composition of Rosins eluted on TC-1 and TC-5 columns

	Rosin 1 (%)			Rosin 2 (%)			Rosin 3 (%)		
Constituent	TC-1	TC-5a	TC-5b	TC-1	TC-5a	TC-5b	TC-1	TC-5a	TC-5b
Pimaric acid ME	-	-		-	-		-	-	
Sandaracopimaric acid ME	11.7	12.8	13.7	12.2	13.7	13.7	11.0	12.3	11.9
Isopimaric acid ME	17.6	17.7	19.2	17.9	18.5	18.7	18.8	18.8	18.5
Palustric acid ME	17.2	17.3	17.4	9.7	9.82	9.9	12.7	13.5	12.5
Dehydroabietic acid ME	15.6	15.2	16.1	27.7	27.0	28.1	11.6	11.2	11.3
Abietic acid ME	24.0	24.1	22.8	17.0	16.2	15.4	33.8	32.4	31.5
Neoabietic acid ME	1.5	1.91	2.1	1.3	2.37	3.8	2.5	2.9	5.1
Merkusic acid ME	12.3	11.0	8.7	14.2	12.4	10.4	9.7	8.7	9.2
	100.0	100.00	100.0	100.0	100.00	100.0	100.0	100.0	100.0

TC-1 and TC-5a: Gradient temperature programme; TC-5b: Isothermal temperature programme
Rosin 1: Sample from East Java, Rosin 2: Sample from West Java, Rosin 3: Sample from North Sumatra

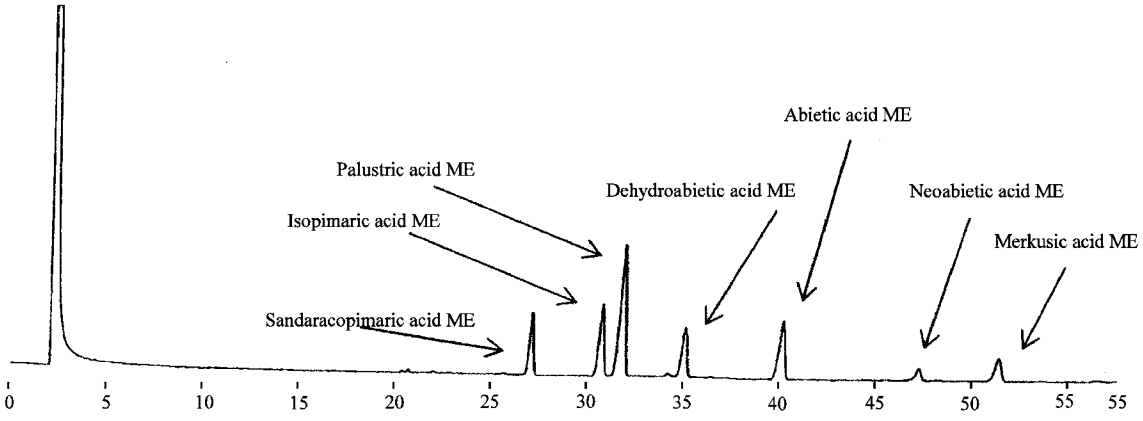

Fig. 5: An example of a GC-FID profile of the acidic fraction and rosin on a TC-5 column (Isothermal temperature programme at 200°C, other conditions similar to the gradient programme)

from similar samples were used[4,5,9,13], both TC-1 and TC-5 columns could not elute levopimaric acid ME and it did not appear on the GC chromatogram. This is the first report that levopimaric acid ME in acidic fractions and rosins of Indonesian *Pinus merkusii* could not be eluted together with palustric acid ME. Moreover, merkusic acid was identified in all samples.

The most common constituents of acidic fractions and rosins are diterpene acids. The palustric acid ME is the major constituent in all acidic fractions (29.0-42.7%), followed by isopimaric acid ME (14.2-20.3%) (Table 3). However, neither samples contain pimaric acid ME, which distinguishes *Pinus merkusii* from other pine species. These results were quantitatively similar to the corresponding data in the literature[4,9]. However, by using the TC-1 column there is some difference in the second constituent in acidic fraction 4 (West Sumatra). This was abietic acid ME (15.73%), not isopimaric acid ME (7.3%). Moreover, the major constituent in rosins was abietic acid ME, especially in rosin 1 (R1) (East Java) and rosin 3 (R3) (North Sumatra), at around 22.8-33.8% (Table 4). While in rosin 2 (R2) (West Java) the major constituent was

dehydroabietic acid (27.0-28.1%) and the second major constituent was isopimaric acid ME similar to the second major constituent in the acidic fractions (Table 4). Abietic acid ME was third. Further, the minor constituent in all analyzed samples was neoabietic acid, in a range of 1.2-5.1%. Moreover, *Pinus merkusii* is unique among pine species in that it contains merkusic acid constituent. Analysis of the acidic fractions and rosins of *Pinus merkusii* in the samples showed that merkusic acid content ranged from 8.3-14.6%. This was quite consistent with the data published elsewhere[4,5,9,13] and was still higher than the amount of merkusic acid in oleoresin of *Pinus latteri*, a pine species from China[15]. The presence of two carboxylic acid groups in merkusic acid contributes to an exceptionally high acid number in the rosin of *Pinus merkusii*[13]. Furthermore, the presence of merkusic acid in the resin further distinguishes *Pinus merkusii* from other pine species.

As described above, all acidic fractions mainly contained palustric acid ME and almost all rosin samples primarily contained abietic acid ME. However, there was a significant difference in content, with the amount of palustric acid ME greater in the acidic fraction than in rosin. By contrast, levels of abietic acid and dehydroabitetic acid were lower. Moreover, based on the locations of merkus pine stands, the difference in sites appears to follow a pattern according to where the samples were from, with the proportion of palustric acid ME in acidic fractions increasing from east to west (Fig. 1, Table 3 and 4). From Java to Sumatra it tends to be more humid, in the west, where there is much more rain. Furthermore, genetic factors probably play a major role in determining both the yield and composition of the resins[16]. This will influence the quality of pine gum oleoresins as raw materials for distillation in the Rosin and Turpentine Industries. Therefore, the difference in the content of major constituents among the acidic fractions could be attributed to the ecological and climatic conditions of the region, as well as genetic factors.

In conclusion, acidic and neutral fractions made up about 13-23 and 66-79%, respectively, of pine oleoresin samples obtained from several Rosin and Turpentine Industries in Indonesia. The GC and GC-MS analyses indicated that the neutral fraction and turpentine oils mostly comprised Δ-3-carene and β-pinene with α-pinene the major component. While determining the chemical composition of acidic fractions and rosins from Indonesian *Pinus merkusii*, we found that relative retention time could not be used to identify each acid. Based on the mass spectral comparison of acid fractions and rosins, the main constituents were sandaracopimaric acid ME, isopimaric acid ME, palustric ME, dehydroabietic acid ME, abietic acid ME, neoabietic acid ME and merkusic acid ME. Palustric acid ME was the most abundant in acidic fractions; and abietic acid ME in rosins. Neither TC-1 nor TC-5 column could elute levopimaric ME together with palustric acid ME. Acidic fractions and rosins of Indonesian *Pinus merkusii* do not contain levopimaric acid in palustric acid ME.

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