Chapter 7
Resolution of Complex Sesquiterpene Hydrocarbons in *Aquilaria malaccensis* Volatile Oils Using Gas Chromatography Technique

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Abstract Agarwood or *gaharu* is resin-impregnated wood of the tree genus *Aquilaria* (Thymelaeaceae). In Malaysia, the main agarwood producer is *Aquilaria malaccensis* and oil extracted from this species is highly priced. One of the challenges in commercializing agarwood is the lack of universal standard to classify the aromatic oils. Our present knowledge places the main aromatic compounds of agarwood oil in the sesquiterpene hydrocarbon region. In this work, we extracted agarwood oil using hydrodistillation method in the laboratory and compared with a commercial-scale extraction in the factory. We analyzed the sesquiterpene hydrocarbon region using several highly sophisticated detection systems. Using GC-FID, 12 sesquiterpene hydrocarbons were identified, while another eight were determined using GC-MS. Five compounds were identified in both analytical techniques: aromadendrene, α-bulnesene, α-guaiene, γ-gurjunene, and β-maaliene. Advanced analysis using GC×GC/TOFMS detected 24 sesquiterpene hydrocarbons in both laboratory and pilot scale agarwood oils. Many of the sesquiterpene hydrocarbons identified provide the woody aroma to the agarwood oil. Specifically, α-gurjunene and α-guaiene contribute to the woody balsamic aroma, while α-copaene contributes to the spicy-wood aroma. In total, 33 sesquiterpene hydrocarbons were identified from *A. malaccensis* in the present study, with high certainty. Results from this study can be used toward establishing a universal standard for agarwood oil from the genus *Aquilaria* in the global market, which is presently lacking.

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7.1 Introduction

Natural agarwood is the pathological product from diseased *Aquilaria* tree primarily because of wounds on the trunk. Consequently, the tree produces a type of resin (known as agar, agarwood, or *gaharu*) that is both high in volatile organic constituents and fragrant as a response to the attack. It is believed that the fragrant resin assists the tree in suppressing or retarding the microbial growth. The affected wood became dark brown or black due to the increased mass and wood density from resin development, leaving the unaffected wood in its original pale beige color. The high-quality agarwood is normally recognized from its dark color and strong aroma. The term black agar refers to the agarwood that resembles black stone as indicated in Fig. 7.1. Agarwood is primarily used as incense, while its essential oil is in heavy demand in the perfume industry as evidenced from the recent expansion of new consumer products such as agarwood essence, soap, and shampoo.

Several methods have been used to classify the physical properties of agarwood for trade purposes. For instance, brown agarwood without any black tone is often used as incense. Meanwhile, yellow wood with interspersed bands of brown or black resin is graded as the lowest quality and is commonly distilled for the oil (Chang et al. 2002). Therefore, low-cost agarwood oil can be obtained to be utilized in toiletries or perfumeries or blended as carrier oils. At present, there is very little information on the quality of different agarwood essential oils produced.

Essential oils, otherwise known as essences or volatile oils, are the volatile of secondary metabolites produced by plants either for their nutrition or other purposes (e.g. as protectant or attractant). The "essential oil" term is often used to illustrate the complex mixtures of chemical components in plants generally extracted using steam distillation, solvent extraction, or physically pressed plant material techniques (Ernest 1948; Yeung 1980). Their chemical constituents are widely utilized for their fragrance, flavor, pharmacological, antimicrobial, insect repellency, and other medicinal properties (Bakkali et al. 2008; Burfield and Reekie 2005; Burt 2004; Edris 2007).

Fig. 7.1 A high-quality natural agarwood often has a dark and dense resinous appearance

Minor differences in these oil compositions can significantly alter their odor or flavor. Therefore, to ensure consistency of the products manufactured over time, the extraction process and composition analysis must be done precisely. Often time, the identification of numerous components in essential oils has been very challenging due to their complex mixture (Adams 2001). Nowadays, several analytical techniques have been developed to solve separation and identification of complex mixture compounds in essential oils for instant enantioseparation. Therefore, this chapter intends to explore the resolution mixture of sesquiterpene hydrocarbons in high-quality agarwood oil using gas chromatography (GC) and advanced two-dimensional GC system.

7.2 Hydrodistillation for Agarwood Essential Oil Extraction

There are several methods of essential oil extraction that have been applied in research laboratories and industries such as the hydrodistillation, microwave-assisted, and supercritical fluid extraction methods. Each method has its own advantages and disadvantages in relation to the quality of the extracted oil such as the yield, duration, cost, and ability to extract targeted compounds (Augusto et al. 2003; Boris 2005; Luque de Castro et al. 1999).

However, hydrodistillation is the most popular method for extracting agarwood essential oil in commercial scale due to easy operation, lower cost, and green water-based process. The copper distillation pot has been traditionally used and it is still in use by some traders. Nowadays, the copper has been replaced by stainless steel. In this method, the pot is filled with fermented agarwood chips or powder and then heated on a brick furnace (Fig. 7.2a). The heating process takes a long time, possibly 2–3 continuous days to ensure all the oil has been extracted. The hot vapor then goes through a steel condenser, where external running water jacket cools the vapors,

Fig. 7.2 A commercial hydrodistillation system for agarwood oil extraction in Gua Musang, Kelantan (Malaysia). (**a**) Extraction contraption and (**b**) extracted oil in the collecting cone

which then drop into a collecting separating funnel where oil separates from water gravitationally (Fig. 7.2b) (Burfield and Kirkham 2005).

Meanwhile, laboratory scale hydrodistillation technique utilizes a Clevenger-type apparatus in which the raw material is heated to reflux as illustrated in Fig. 7.3. The essential oil components tend to form an azeotropic mixture with water during this process, and vapors containing volatile constituents are carried along with evaporated steam to a condenser. Prior to the extraction, distilled water is introduced into Clevenger apparatus with 5 ml of analytical grade hexane or other nonpolar solvents via the inlet. At the end, the mixture of essential oil and hexane is collected and mixed with anhydrous sodium sulfate to remove water content. Essential oil then is purified by passing through inert gas usually nitrogen.

The laboratory hydrodistillation period can take from 3 to 24 h depending on the sample. Distillation temperatures should be 100 °C; however, the biomass movement in the pot and heat distribution can vary and produce temporary high temperature, which in turn can lead to formation of artifacts. A well-regulated operation system can help in reducing compound rearrangement and terpene decomposition. Oil of poor quality can also be produced as the result of prolonged heating in contact with water (Stewart 2005). The polymerization of aldehydes, hydrolysis of esters, or decomposition (e.g., dehydration) of volatile components commonly occurs during this stage.

Fig. 7.3 Hydrodistillation using Clevenger-type apparatus

7.3 Components of Volatile Agarwood Oil

Agarwood contains several types of sesquiterpenes, and it has become the subject of active research in the past 40 years or more. It is believed that Agarol was the first sesquiterpene isolated from agarwood (Bhattacharyya et al. 1952; Maheshwari et al. 1963; Bucchi and Wuest 1979). Nowadays, more than 70 sesquiterpene compounds have been identified from agarwood (Naef 2011). The basic molecule of terpenes is the isoprene unit C_5H_8. Chemically, the terpenes can be divided into several classes including mono-, sesqui-, and diterpenes. Monoterpenes are comprised of two isoprene units, $C_{10}H_{16}$, whereas sesquiterpenes, $C_{15}H_{24}$, contained three isoprene units. Sesquiterpenes that are important in agarwood oils are sesquiterpene hydrocarbons ($C_{15}H_{24}$) and oxygenated sesquiterpenes ($C_{15}H_{26}O$). They are derived from the sesquiterpene skeletons and grouped as agarofuran, agarospirane/vetispirane, cadinane, eremophilane/valencane, eudesmane/selinane, guaiene, and prezizane types (Fig. 7.4). Important oxygenated sesquiterpenes including agarospirol, jinkohol, jinkohol-eromol, and kusenol that may contribute to the characteristic woody aroma of agarwood are shown in Fig. 7.5.

Generally, agarwood oils are mixtures of sesquiterpenes, oxygenated sesquiterpenes, oxygenated phenyls, carboxylic, and carbonyl hydrocarbons. Table 7.1 shows the main sesquiterpene hydrocarbons and oxygenated sesquiterpenes, which have been identified by previous agarwood research.

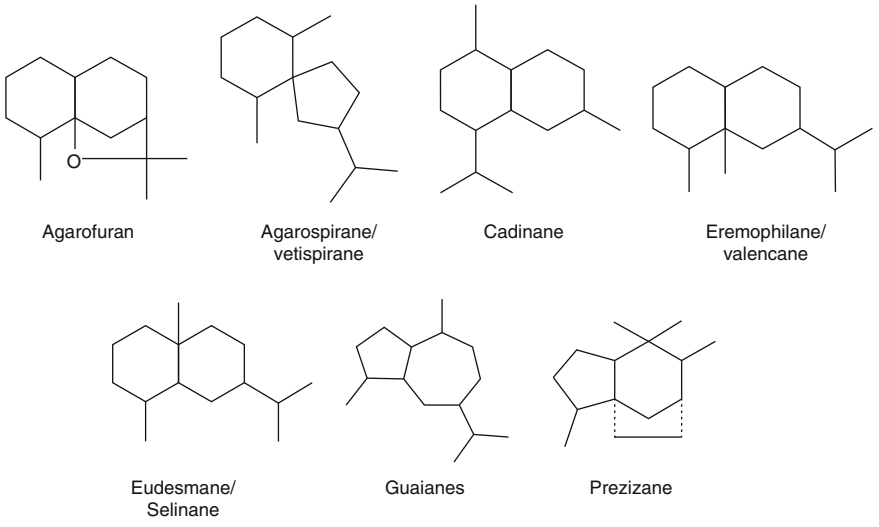

Fig. 7.4 Skeleton of hydrocarbons found in volatile agarwood oil (Source: Naef (2011), Chen et al. (2012))

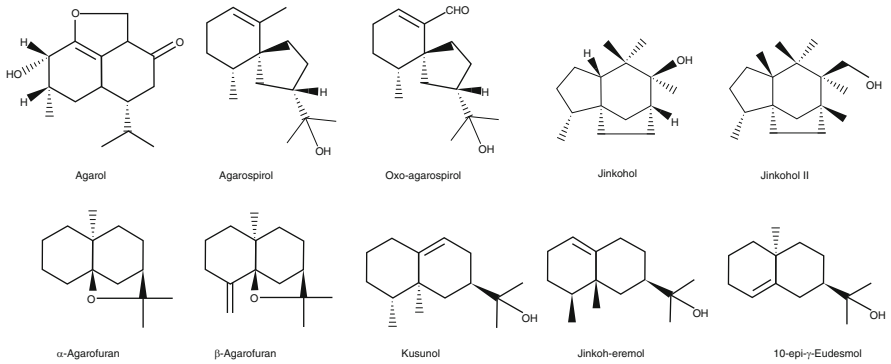

Fig. 7.5 Skeleton of oxygenated sesquiterpenes found in volatile agarwood oil (Source: Maheshwari et al. (1963), Varma et al. (1965), Nakanishi et al. (1981, 1984), Ishihara and Tsuneya (1993a, b))

7.4 Compound Detection via Gas Chromatography (GC)

GC is a well-known analytical technique utilized for separation of volatile compounds. It provides qualitative and quantitative information for each compound present in the sample. Initially, the solid or liquid sample is vaporized into a gaseous state by heating process. As the vaporized sample moves through a GC column, these compounds are fractioned between a mobile phase (gas) and a stationary phase (solid or liquid). The compounds are separated in time and space due to the differential fractionation of the solute into the stationary phase.

GC finds its main application in the analyses of essential oils, fatty acids, mono- and sesqui-terpenes, and sulfur compounds (Arian 2002; Bartle and Myers 2002). An inert carrier gas under pressure (helium, argon, nitrogen, or hydrogen) is used to convey the vaporized sample through a narrow column. Normally, GC columns are made from fused silica tubes with internal diameters between 0.1 and 1 mm. To enhance thermal stability, 0.1–5 μm stationary phase films are bound and cross-linked to the column's inner surface. The column then is stationed inside an oven with temperature control. This allows the column to be heated slowly from an ambient temperature up to 350–450 °C for separating the chemical compounds.

Flame ionization detector (FID) and mass spectrometer detector (MSD) are the most commonly used instruments for detection of eluting compounds in GC. The applicability of GC with "universal" detectors makes this analytical instrument one of the most suitable option for essential oil study. GC has three main advantages over other analytical methods; it is very rapid, it has a very high separation capacity, and it is highly sensitive (König et al. 1999).

Table 7.1 List of sesquiterpenes from agarwood oils of four *Aquilaria* species from different countries and extraction mode

Aquilaria species	Extraction mode	Main constituents	Reference
A. agallocha from:			
Bangladesh (Sylhet)	Hydrodistillation	Aristolene; caryophyllene oxide; γ-eudesmol; β-selinene; valencene	Bhuiyan et al. (2009)
India	Petroleum ether	α-Agarofuran; β-agarofuran; *nor*-ketoagarofuran; 3,4-dihydroxydihydro-agarofuran	Maheshwari et al. (1963)
Indonesia (type B)	Benzene	Jinkohol II	Nakanishi et al. (1983)
Indonesia (type B)	Benzene	Agarol; oxo-agarospirol; (-)-10-*epi*-eudesmol	Nakanishi et al. (1984)
Vietnam (Kyara, Kanankoh)	Acetone	Selina-3,11-dien-9-one; selina-3,11-dien-14-al	Ishihara et al. (1991a)
Vietnam (Ryoku-yu, Kanakoh)	Acetone	(+)-Guaia-1(10),11-dien-15, 2-olide; (-)-guaia-1(10),11-dien-15-ol; rotundone	Ishihara et al. (1991b)
Vietnam (type A)	Benzene	β-Agarofuran; *nor*-ketoagarofuran; agarospirol; oxo-agarospirol; dihydrokaranone; jinkoh-eremol; kusunol	Yoneda et al. (1984)
A. crassna from:			
Thailand	Hydrodistillation, supercritical CO_2	Agarospirol; γ-eudesmol; 10-*epi*-γ-eudesmol; epoxy bulnesene; kusunol; selina-3,11-dien-9-one; selina-3,11-dien-14-al; γ-selinene;	Wetwitayaklung et al. (2009)
Thailand (cultivated, Bo Rai in Trat province)	Hydrodistillation (pretreatment with enzyme), supercritical CO_2	Agarospirol; caryophyllene oxide; eudesma-4,11-diene-3-one; 10-*epi*-γ-eudesmol; guaia-3,9-diene; β-guaiene; selina-3,7(11)-diene; δ-selinene	Yoswathana (2013)
Thailand (cultivated, Bo Rai in Trat province)	Hydrodistillation with different pretreatment, subcritical water extraction	Agarospirol; aristol-9-dien-8-one; eudesma-4,11-dien-3-one; guaia-3,9-diene; α-guaiene; β-guaiene; nootkatone; selina-3,7(11)-diene	Yoswathana et al. (2012)
Thailand (Trat province)	Hydrodistillation	α-Agarofuran; agarospirol; aromadendrene epoxide; aristolene; α-gurjunene; 10-*epi*-γ-eudesmol	Pornpunyapat et al. (2011)

(continued)

Table 7.1 (continued)

Aquilaria species	Extraction mode	Main constituents	Reference
A. malaccensis from:			
India (Hojai in Assam province)	Hydrodistillation	Aromadendrene; (+)-calarene; 6-guaiadiene; valencene	Jayachandran et al. (2014)
Indonesia (type B)	Benzene	Jinkohol	Nakanishi et al. (1981)
Indonesia (type B)	Benzene	Jinkohol II	Nakanishi et al. (1983)
Indonesia (type B)	Benzene	Agarol, 3,4-dihydroxydihydro-agarofuran, oxo-agarospirol, jinkoh-eremol	Nakanishi et al. (1984)
Malaysia (grade C)	Hydrodistillation	α-Agarofuran; β-agarofuran; *nor*-ketoagarofuran; agarospirol; 3-phenyl-2-butanone; β-eudesmol; 10-*epi*-γ-eudesmol; α-guaiene; jinkoh-eremol; jinkohol II; kusunol	Nor Azah et al. (2008)
Vietnam (Kyara, Kanankoh)	Acetone	Oxo-agarospirol, α-guaiene, (-)-Guaia-1(10),11-dien-15-al	Ishihara et al. (1991a)
Vietnam (type A)	Benzene	α-Agarofuran, 3,4-dihydroxydihydro-agarofuran, oxo-agarospirol, jinkoh-eremol, jinkohol, jinkohol II, kusunol	Yoneda et al. (1984)
A. sinensis from			
China	Hydrodistillation	Agarospirol; caryophyllene oxide; α-copaen-11-ol; eremophila-7(11),9-diene-8-one; eudesm-7(11)-en-4α-ol; γ-eudesmol; guaia-1(10),11-dien-9-one; guaiol; selina-3,11-dien-14-al; α-selinene	Chen et al. (2011)
China (Hainan, Guangdong)	Ethanol, hydrodistillation	Agarospirol; aristolene; caryophyllene oxide; α-copaen-11-ol; eudesm-7(11)-en-4α-ol; γ-eudesmol; guaiol	Xing et al. (2012)

Source: Tajuddin (2010), Naef (2011)

7.4.1 Gas Chromatography-Flame Ionization Detector (GC-FID)

GC-FID is a common technique to identify compounds by comparison of retention time (t_r). Those retention times are converted into Kovats retention index (I), which is a system-independent constant. The I values are actually the normalized of t_r value for adjacently eluting n-alkanes with those t_r values obtained from chromatogram. Parameters such as film thickness and diameter, column length, void time, carrier gas velocity, and pressure influenced the t_r values. However, derivation of I values is relatively independent, thus allowing the comparison of values calculated under various conditions and different analytical laboratories. Joulain and König (1998) suggested that by comparing experimental I values with those known values, Kovats indices can be used to identify chemical compounds in the samples.

The sensitive detection of flame ionization detector (FID) toward molecules with carbon-hydrogen bonds makes it the most utilized detector in GC. The low limit of detection ranging from 0.1 ppm to almost 100 % is highly preferable for hydrocarbon analysis. FID responds poorly to compounds such as CCl_4, H_2S, and NH_3 and in some cases not at all. Nonetheless, changing the carrier gas flow rate only has small impact on detector response. As a matter of fact, FID is not concentration sensitive, but it is sensitive to mass of hydrocarbons. The stable response rate also resulted in FID being more resistant to contamination either by column bleed or sample itself. Although it is a user-friendly and robust instrument, the sample is destroyed during analysis as compound ionization process uses hydrogen diffusion flame.

7.4.2 Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS is literally known as a hyphenated analytical technique as it combines two techniques, namely, GC and mass spectrometry (MS) to form a powerful single method for chemical compound analysis including essential oils. This combination is very useful for qualitative and quantitative evaluation of known and unknown sample as GC works to separate the component of a solution and MS characterizes each compound individually.

Mass spectrometry detection is based on analysis of generated charged particles (ions) from molecules of the analytes. This technique provides details regarding their molecular weight and chemical structures. Generally, various MS detector types and sample introduction techniques enable a broad application for sample analysis. According to Gross (2004), there are three specific regions for MS instrumentation; ionizer, ion analyzer, and detector.

7.4.3 *Comprehensive Two-Dimensional Gas Chromatography (GC×GC)*

GC×GC technique nowadays is utilized for its capability to analyze very complex samples (Marriott et al. 2001; Beens and Brinkman 2005; Adahchour et al. 2006; Mondello et al. 2007). This analytical tool is so powerful that it is extensively used to deal with separation and resolution issues in conventional GC. Giddings (1984) was the first person known to discuss and elucidate many basic requirements of "separation dimension" in his revolutionary paper on chromatography techniques. A single column for separation based on particular parameters (stationary phase, length, size, etc.) represents a separation dimension. Therefore, the term "GC×GC" indicates that two different columns are employed to create a two-dimensional separation for the sample. In fact, separation process in these columns is orthogonal and independent of each other. Despite the fact that the concept of GC×GC separation was first suggested by Giddings as a possibility, the initial practical implementation was demonstrated in 1991 (Marriott and Shellie 2002).

The extensive implementation and rapid improvement of GC×GC primarily contributed by its significant enhancement in peak capacity, sensitivity, resolution, as well as selectivity of the separation, aside for well-ordered and highly structured chromatograms. Originally, GC×GC was mainly utilized for petroleum products analysis. However, forensic science and food and fragrance industry have also reported to successfully use of GC×GC for respective studies (Dimandja et al. 2000; Micyus et al. 2005; Mondello et al. 2005, 2007; Khummueng et al. 2006; Min et al. 2006; Ma et al. 2007; Rocha et al. 2007; Cardeal and Marriott 2009; Pripdeevech et al. 2010). According to Schoenmakers et al. (2000), the formerly unachievable results using one dimension (1DGC), regardless if it was combined with the most powerful MS detectors, can be obtained through GC×GC analysis.

7.5 A Case Study of Essential Oil Analysis of Agarwood from Peninsular Malaysia

We conducted this study using the same source of agarwood samples, namely, *A. malaccensis*. Both infected woods used in the laboratory and commercial-scale hydrodistillation originated from the Kelantan Forest.

7.5.1 *Essential Oil Extraction*

Parameters used in the extraction procedures and characteristics of the oil produced are listed in Table 7.2. Prior to the extraction, wood samples were grinded into sawdust to maximize surface area and thus enhancing oil yield.

Table 7.2 Comparisons between oils extracted using laboratory and commercial-scale methods

	Laboratory	Commercial
Raw material, kg	0.1	25
Duration of extraction, h	12	36
Yield, %	0.2	0.2
Optical rotation, $[\alpha]^{25}$	−0.1065	−0.1084
Odor characteristic	Sweet, woody, aromatic	Sweet, woody, aromatic
Color	Greenish	Dark green

7.5.2 Compound Analysis Using GC-FID and GC-MS

GC-FID analysis was performed using an Agilent 7890A Network System gas chromatography, while GC-MS with the same system was attached to a mass spectrometer (Agilent 5975C) with detector in full-scan mode under electron impact ionization (EI, 70 eV). A capillary column (DB-1 ms 30 m × 0.25 mm I.D.; 0.25 μm film thickness) purchased from J&W Scientific (Folsom, California) was chosen for the compound analysis by GC-FID and GC-MS. The oven temperature was programmed for 60 °C for 1 min and then ramped at 3 °C/min to 250 °C and held for 10 min. Injector inlet and detector temperatures were set at 250 °C, while sample injection (1 μL) was set at split ratio 1:5.

Over 36 components have been identified from the oil samples (Table 7.3). The laboratory oil had 36.7 % carboxylic acid derivatives, 18 % sesquiterpene hydrocarbons, and 31.9 % oxygenated sesquiterpenes, while the commercial oil had 3.4 %, 9.4 %, and 34.6 %, respectively. We identified eight sesquiterpene hydrocarbons in both oil samples (Table 7.3). Major sesquiterpene hydrocarbons in the laboratory oil were α-guaiene (5.8 %), β-selinene (4.9 %), and α-muurolene (3.4 %) and in the commercial oil α-guaiene (2.8 %) and α-bulnesene (2.8 %) (Figs. 7.6 and 7.7).

7.5.3 Compound Analysis Using Comprehensive GC × GC System

An Agilent 6890 GC, a Pegasus IV time-of-flight mass spectrometer (LECO Corp., St. Joseph, MI, USA), and a cold-jet modular KT-2001 Retrofit prototype (Zoex Corp., Lincoln, NE, USA) were used for GC × GC analysis. The first column was a nonpolar DB-1 ms (30 m × 0.25 mm I.D.; 0.25 μm film thickness) and the second column was a DB-Wax (1.0 m × 0.10 mm I.D.; 0.10 μm film thickness). Both columns were purchased from J&W Scientific (Folsom, California). Helium was used as carrier gas with head pressure at 37 atm. The initial temperature of the first dimension was maintained at 60 °C for 1 min, and the subsequent temperature program was ramped at a rate of 3 °C min^{-1} until 220 °C where it was held isothermally for 10 min. Meanwhile, the initial temperature of the second dimension was 75 °C,

Table 7.3 Chemical composition of volatile agarwood oils based on gas chromatography analysis

Compounds	DB1 column	Laboratory-extracted oil[a]	Commercial hydrodistilled oil[a]	Identification[b, c]
Carboxylic acid derivatives				
Benzaldehyde	935	3.3	–	RI, MS
2-Hydroxy-benzaldehyde	1003	0.6	–	RI, MS
Acetophenone	1066	0.7	–	RI, MS
4-Phenyl-2-butanone	1210	32.1	3.4	RI, MS
Total, %		**36.7**	**3.4**	
Sesquiterpene hydrocarbons				
β-Maaliene	1414	0.4	0.7	RI, MS
α-Guaiene	1440	5.8	2.8	RI, MS
Aromadendrene	1443	–	0.6	RI, MS
γ-Gurjunene	1472	0.7	1	RI, MS
β-Selinene	1486	4.9	–	RI, MS
α-Muurolene	1496	3.4	0.7	RI, MS
γ-Guaiene	1499	1.5	0.8	RI, MS
α-Bulnesene	1503	1.3	2.8	RI, MS
Total, %		**18**	**9.4**	
Oxygenated sesquiterpenes				
α-Elemol	1530	–	3.3	RI, MS
Caryophyllene oxide	1600	0.9	8.6	RI
Guaiol	1603	0.6	1.2	RI
Humulene epoxide II	1606	1.7	2.3	RI
1,5-Epoxy-*nor*-ketoguaiene	1614	1.1	0.6	RI, MS
10-*epi*-γ-Eudesmol	1619	1.6	0.8	RI, MS
Agarospirol	1631	0.9	1.4	RI, MS
epi-α-Cadinol	1640	–	2.9	RI
Jinkoh-eremol	1643	6.5	0.5	RI
Kusunol	1650	1	0.6	RI
α-Eudesmol	1652	0.7	0.9	RI, MS
Bulnesol	1664	1.5	0.6	RI, MS
Dehydrojinkoh-eremol	1673	1.4	1.2	RI, MS
epi-α-Bisabolol	1678	1.5	1	RI
α-Bisabolol	1683	1.8	0.5	RI
Selina-3,11-dien-9-one	1687	1.3	0.5	RI, MS
Rotundone	1703	0.5	–	RI, MS
Guaia-1(10),11-dien-15-al	1806	1.7	–	RI, MS
Guaia-1(10),11-dien-15-oic acid	1811	0.6	–	RI, MS
Karanone	1812	–	1.1	RI, MS
Oxo-agarospirol	1822	0.3	0.8	RI, MS

Table 7.3 (continued)

Compounds	DB1 column	Laboratory-extracted oil[a]	Commercial hydrodistilled oil[a]	Identification[b, c]
Eudesmol	1880	2.1	3.2	RI, MS
n-Hexadecanoic acid	1948	4.2	2.1	RI
Guaia-1(10),11-dien-15,2-olide	2019	–	0.5	RI, MS
Total, %		**31.9**	**34.6**	

Source: Tajuddin (2010)

[a]Components are listed in order of their relative content >0.1 %

[b]RI, linear retention indices were determined relative to the retention times on a DB-1 column of a homologous series of C_8-C_{20} *n*-alkanes

[c]MS, identification by comparison of the MS with those of the NIST library (>90 % match from the library)

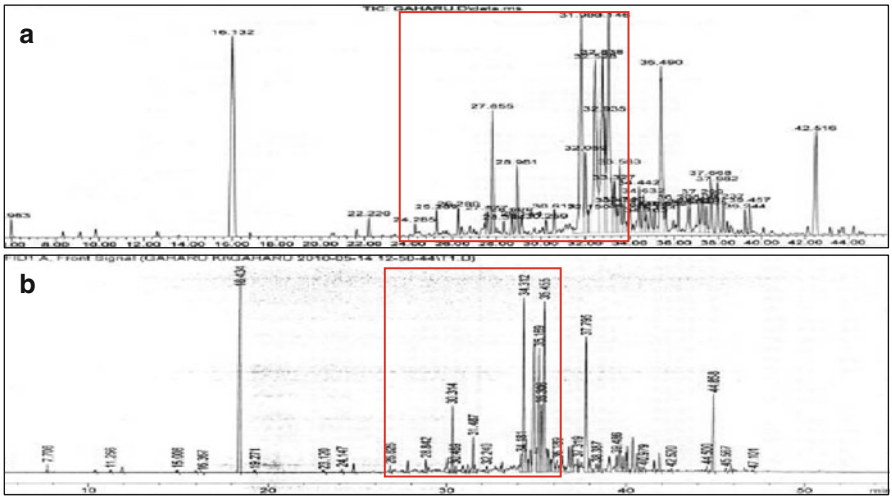

Fig. 7.6 Sesquiterpene hydrocarbon positions in (**a**) GC-FID and (**b**) GC-MS chromatograms, for laboratory oil

held for 30s, and then heated at 5 °C min^{-1} until 230 °C and again held isothermally for 10 min. Peak identification was made using TOFMS with electron impact ionization.

Eighteen sesquiterpene hydrocarbons were identified in the laboratory oil (Table 7.4, Fig. 7.8a) and 12 in the commercial oil (Table 7.5, Fig. 7.8b), bringing to a total of 24 in both oil samples (Table 7.6). Major sesquiterpene hydrocarbons in the laboratory sample were α-bulnesene (15.7 %), α-caryophyllene (13.61 %), and α-guaiene (11.31 %), whereas the commercial sample was dominated by aromadendrene (27.7 %), β-cubebene (9.5 %), and α-guaiene (8.7 %).

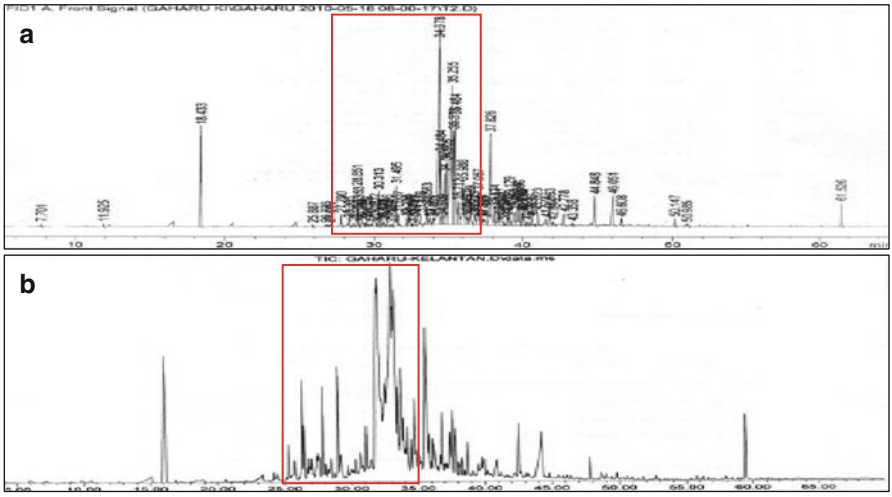

Fig. 7.7 Sesquiterpene hydrocarbon positions in (**a**) GC-FID and (**b**) GC-MS chromatograms, for commercial oil

7.5.4 General Discussion

Sesquiterpene hydrocarbons in agarwood oil are poorly studied although they are main contributors to the aroma arising from plant materials. For example, β-elemol is a fragrant sesquiterpene, which contributes to peppery and lemony odors. Other sesquiterpene like β-caryophyllene contributes to sweet, spicy, and fruity odors (Breitmaier 2006). A total of 27 sesquiterpene hydrocarbons were identified using GC-FID, GC-MS, and advance analysis via GC×GC/TOFMS (Table 7.7). GC-MS and GC-FID analyses found only seven to eight sesquiterpene hydrocarbons in the agarwood oil samples, whereas GC×GC/TOFMS was detected between 12 and 18. Most of these compounds also exhibit a specific odor unique to agarwood oil (Table 7.8).

Commercial oil contains lesser content of carboxylic acid derivatives in comparison to laboratory oil and is due to vaporization during the prolonged extraction time of 3 days (Tajuddin and Yusoff 2010). Previously, four compounds have been reported from agarwood, β-gurjunene (Ishihara and Tsuneya 1993a; Wetwitayaklung et al. 2009), γ-selinene (Wetwitayaklung et al. 2009), α-guaiene (Ishihara et al. 1991b), and α-bulnesene (Ishihara and Tsuneya 1993a; Wetwitayaklung et al. 2009), similar to our findings.

Due to extensive isomerization, sesquiterpene hydrocarbons tend to present themselves as a complex mixture in many types of essential oil analyses (Shellie et al. 2001). Conventional techniques such as GC-MS are not efficient in resolving such a complex mixture of sesquiterpene hydrocarbons (Fig. 7.9a). Improvements to the multidimensional gas chromatography (GC×GC) have enabled resolution to

Table 7.4 Sesquiterpene hydrocarbons identified in the laboratory oil via GC×GC/TOFMS

Peak	Compounds	R.T. (s)	Similarity	Reverse	Probability	Area %	CAS
1	Isoledene	990, 2.060	801	801	1824	0.22	95910-36-4
2	α-Copaene	980, 1.990	909	911	4590	0.35	3856-25-5
3	Selina-3,7(11)-diene	1335, 2.570	648	651	3712	0.21	6813-21-4
4	δ-Cadinene	1305, 2.460	850	856	3435	1.74	483-76-1
5	α-Guaiene	1265, 2.470	934	934	4680	11.31	3691-12-1
6	*allo*-Aromadendrene	1240, 2.480	885	887	1678	2.46	25246-27-9
7	α-Selinene	1225, 2.400	884	898	1939	3.82	473-13-2
8	α-Muurolene	1210, 2.360	895	901	4741	2.49	31983-22-9
9	α-Himachalene	1160, 2.330	803	831	1410	0.22	3853-83-6
10	α-Caryophyllene	1150, 2.360	930	934	7663	13.61	6753-98-6
11	Aromadendrene	1145, 2.330	847	857	1839	10.28	109119-91-7
12	β-Patchoulene	1130, 2.250	901	908	3358	5.64	514-51-2
13	α-Bulnesene	1115, 2.240	947	950	3124	15.69	3691-11-0
14	Germacrene D	1100, 2.230	820	865	1329	0.26	23986-74-5
15	β-Caryophyllene	1075, 2.260	929	929	3250	1.19	87-44-5
16	β-Sesquiphellandrene	1065, 2.230	883	898	1060	0.62	20307-83-9
17	*trans*-α-Bergamotene	1065, 2.110	870	875	4787	2.04	13474-59-4
18	di-*epi*-α-Cedrene	1035, 2.140	879	881	1140	0.14	50894-66-1

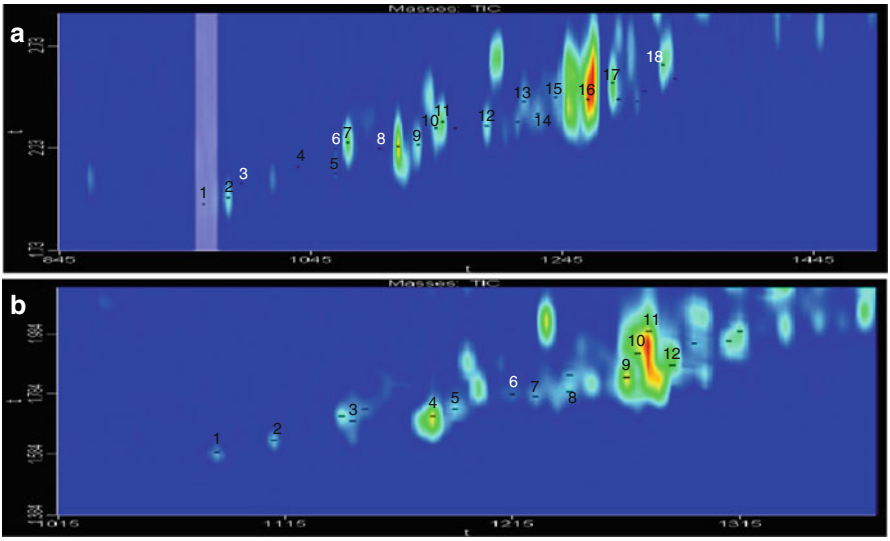

Fig. 7.8 Partial 2D GC×GC/TOFMS chromatogram for the (**a**) laboratory and (**b**) commercial oils. Labeled compounds correspond to the respective peaks listed in Tables 7.4 and 7.5

Table 7.5 Sesquiterpene hydrocarbons identified in the commercial oil via GC×GC/TOFMS

Peak	Compounds	R.T. (s)	Similarity	Reverse	Probability	Area %	CAS
1	α-Copaene	1085, 1.592	919	921	5087	0.94	3856-25-5
2	β-Elemene	1110, 1.632	900	900	6237	1.25	33880-83-0
3	α-Cedrene	1140, 1.712	911	914	5092	4.45	469-61-4
4	β-Caryophyllene	1150, 1.736	903	903	2881	0.87	87-44-5
5	α-Guaiene	1180, 1.712	948	948	4351	8.73	3691-12-1
6	β-Patchoulene	1190, 1.736	905	917	4462	1.88	514-51-2
7	α-Longipinene	1215, 1.784	832	847	2210	0.82	5989-08-2
8	*allo*-Aromadendrene	1225, 1.776	807	824	923	2.55	25246-27-9
9	α-Muurolene	1240, 1.792	893	908	4652	4.13	31983-22-9
10	α-Curcumene	1240, 1.848	914	927	9149	4.01	644-30-4
11	Aromadendrene	1270, 1.920	836	836	3395	27.66	109119-91-7
12	β-Cubebene	1285, 1.880	809	829	2904	9.45	13744-15-5

Table 7.6 Sesquiterpene hydrocarbons identified via GC × GC/TOFMS

No.	Compounds	Laboratory oil	Commercial oil	CAS registration
1.	*allo*-Aromadendrene	√	√	25246-27-9
2.	Aromadendrene	√	√	109119-91-7
3.	*trans*-α-Bergamotene	√	–	13474-59-4
4.	α-Bulnesene	√	–	3691-11-0
5.	δ-Cadinene	√	–	483-76-1
6.	α-Caryophyllene	√	–	6753-98-6
7.	β- Caryophyllene	√	√	87-44-5
8.	γ-Caryophyllene	–	–	118-65-0
9.	di-*epi*-α-Cedrene	√	–	50894-66-1
10.	α-Cedrene	–	√	469-61-4
11.	α-Copaene	√	√	3856-25-5
12.	β-Cubebene	–	√	13744-15-5
13.	α-Curcumene	–	√	644-30-4
14.	β-Elemene	–	√	33880-83-0
15.	Germacrene D	√	–	23986-74-5
16.	α-Guaiene	√	√	3691-12-1
17.	α-Himachalene	√	–	3853-83-6
18.	Isoledene	√	–	95910-36-4
19.	α-Longipinene	–	√	5989-08-2
20.	α-Muurolene	√	√	31983-22-9
21..	β-Patchoulene	√	√	514-51-2
22.	Selina-3,7(11)-diene	√	–	6813-21-4
23.	α-Selinene	√	–	473-13-2
24.	β-Sesquiphellandrene	√	–	20307-83-9

the complex mixture (Fig. 7.9b). In such cases, structural identification was attained at high certainty (Wu et al. 2004). The ability of GC×GC to enhance peak capacity when combined with deconvolution power from the TOFMS system greatly improves quantification (Fig. 7.9c).

Our results demonstrate that a higher potential in compound detection and identification can be achieved via GC×GC/TOFMS. Using this approach, the number of detected sesquiterpene hydrocarbons was doubled when compared to GC-FID and GC-MS. Better analyte separation via GC×GC and enhanced sensitivity (full mass range acquisition) of TOFMS detection contribute to superior identification capacity (Marriott et al. 2001; Mondello et al. 2007). GC×GC/TOFMS is also applicable for detection of co-eluted peaks due to its deconvolution algorithm and automated peak acquiring. In addition, duration and complexity of the analysis are reduced with the presence of two different polarity columns and established software library for GC×GC.

Table 7.7 Distribution of sesquiterpene hydrocarbons identified in volatile agarwood oils

No.	Compounds	Laboratory oil			Commercial oil		
		GCFID	GCMS	GC×GC/TOFMS	GCFID	GCMS	GC×GC/TOFMS
1.	*allo*-Aromadendrene	√	–	√	√	–	√
2.	Aromadendrene	–	√	√	–	√	√
3.	*trans*-α-Bergamotene	–	–	√	–	–	–
4.	α-Bulnesene	√	√	√	√	√	–
5.	δ-Cadinene	–	–	√	–	–	–
6.	α-Caryophyllene	–	–	√	√	–	–
7.	β- Caryophyllene	–	–	√	–	–	√
8.	γ-Caryophyllene	–	–	–	–	–	–
9.	di-*epi*-α-Cedrene	–	–	√	–	–	–
10.	α-Cedrene	–	–	–	–	–	√
11.	α-Copaene	–	–	√	–	–	√
12.	β-Cubebene	–	–	–	–	–	√
13.	α-Curcumene	–	–	–	–	–	√
14.	β-Elemene	–	–	–	–	–	√
15.	Germacrene D	–	–	√	–	–	–
16.	α-Guaiene	–	√	√	–	√	√
17.	γ-Gurjunene	–	√	–	√	–	–
18.	α-Himachalene	–	–	√	–	–	–
19.	Isoledene	–	–	√	–	–	–
20.	α-Longipinene	–	–	–	–	–	√
21.	β-Maaliene	√	√	–	–	√	–
22.	α-Muurolene	–	–	√	–	–	√
23.	β-Patchoulene	–	–	√	–	–	√
24.	Selina-3,7(11)-diene	–	–	√	–	–	–
25.	α-Selinene	–	–	√	–	–	–
26.	β-Selinene	–	√	–	–	–	–
27.	β-Sesquiphellandrene	–	–	√	–	–	–

Table 7.8 List of major compounds identified in agarwood oil and their odor characteristics

Compounds	Odor characteristics[a]
allo-Aromadendrene	Wood
Aromadendrene	Wood
trans-α-Bergamotene	Wood
α-Bulnesene	Wood
α-Cedrene	Wood
α-Caryophyllene	Wood
α-Copaene	Wood, spice
α-Guaiene	Wood, balsamic
β-Gurjunene	Wood, balsamic
α-Muurolene	Wood
α-Selinene	Wood

[a]Odor characteristics are based on Terry and Heinrich (1984)

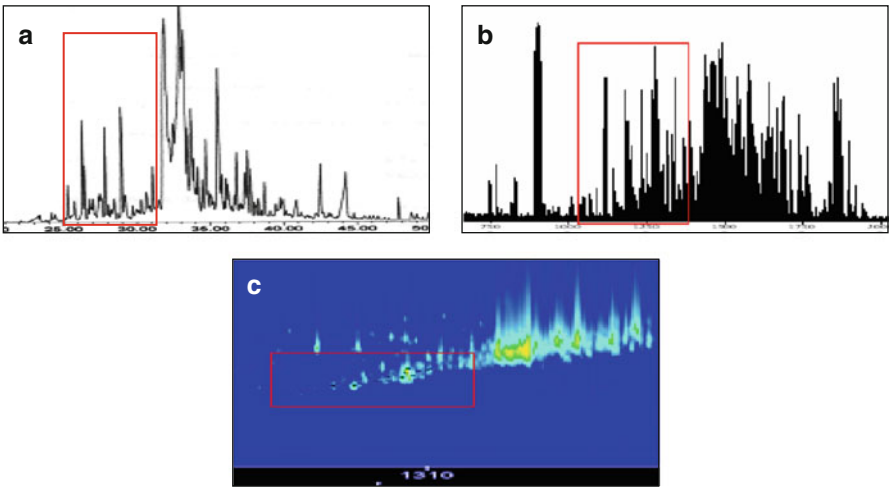

Fig. 7.9 GC×GC/TOFMS has the ability to resolve complex mixtures of sesquiterpene hydrocarbons in volatile agarwood oil. (**a**) GC-MS chromatogram (unable to resolve), (**b**) 1D GC×GC/TOFMS chromatogram (able to resolve), and (**c**) 2D chromatogram GC×GC/TOFMS

7.6 Conclusions

We found GC×GC/TOFMS an effective tool for separating chemical components in very complex mixtures such as in the volatile oils of agarwood, far greater than when using conventional GC-FID or GC-MS. When coupled to TOFMS, the GC×GC system shows a marked improvement in sensitivity and resolution. TOFMS has higher reverse values and similarity compared to MS detector, in addition to its superior mass spectral data in the reference library. This allows identification of a higher number of peaks in the agarwood oils. GC×GC/TOFMS could be adopted as a useful tool to meet demands in agarwood oil grading for trade and forensic purposes.

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