



Does Donglan lacquer tree belong to *Rhus vernicifera* species?

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Received 15 November 2006; received in revised form 23 January 2007; accepted 25 June 2007

Available online 6 July 2007

Abstract

The lacquer trees in Donglan of Guangxi Province, China, were identified totally as the species *Rhus succedanea* found in Vietnam and Taiwan region, based on the results of pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS), an easy and effective method to identify species of trees among those with similar properties. Analyses by IR and NMR, the drying properties, and conventional morphology also confirmed that the Donglan lacquer trees do not belong to *Rhus vernicifera*, like most trees of the China mainland. Some differences, however, such as the enzymatic activity and the components of the lacquer, were found between the Donglan lacquer and the Vietnam lacquer. The Donglan lacquer has a shorter drying time than the latter.

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Keywords: Donglan lacquer; *Rhus succedanea*; Py-GC/MS; Drying properties; Laccol; Urushiol

1. Introduction

Hütermann reported the Chinese had began using lacquer to produce artwork more than 6000 years ago [1], but, in fact, the very famous ZhuQi MuWan (Vermilion Lacquered Wooden Bowl) that was found in 1978 at the Hemudu site in Zhejiang Province, China, was manufactured 7000 years ago, in the Neolithic period [2]. Mayer and Staples [3] indicated that, compared with the laccases of fungi, the laccases of higher plants have only been evaluated and research on the laccases of higher plants is rather scarce, and so that a new investigation on higher plants, especially lacquer trees as one of Chinese peculiar resources, is more urgent.

Lacquer trees are roughly divided into three groups according to their geographic distribution in the world, i.e., *Rhus vernicifera* (mainly distributed in China, Japan, and Korea), *Rhus succedanea* (mainly distributed in north Vietnam and

Taiwan region), and *Melanorrhoea usitata* and *Melanorrhoea laccifera* (mainly distributed in Burma and Laos, and Thailand and Cambodia, respectively). They belong to the family of anacardiaceae that has been proved to produce laccases. They are well documented [4–9] in China and Japan and the dominating distributions in the triangular region ranging from west to Thimbu, Bhutan, northeast to Hatinohe, Japan, and south to Ho Chi Minh, Vietnam are shown in Fig. 1. A circular region centered at Maoba in Hubei Province, China (approximately east longitude 109°, north latitude 30°) is the most important exporter of raw lacquer or Chinese lacquer in the world, and the lacquer is known for its excellent quality.

A latex material exuded from the cut phloem of lacquer trees is collected to obtain lacquer sap and simply filtered according to the traditional Chinese method. The saps from *Rhus vernicifera* and *Rhus succedanea* contain urushiol (60–70%) and laccol (42–44%), water (20–25%) and (32–39%), plant gelatin (5–7%) and (16–17%), respectively, polysaccharides (2–5%), and glycoprotein (3–7%), *Rhus* laccases (~1%) and other components [10–15]. Urushiol and laccol both play main roles in lacquer sap and lacquer film under catalysis by *Rhus* laccases. Thus, in this study, a simple method using pyrolysis-gas

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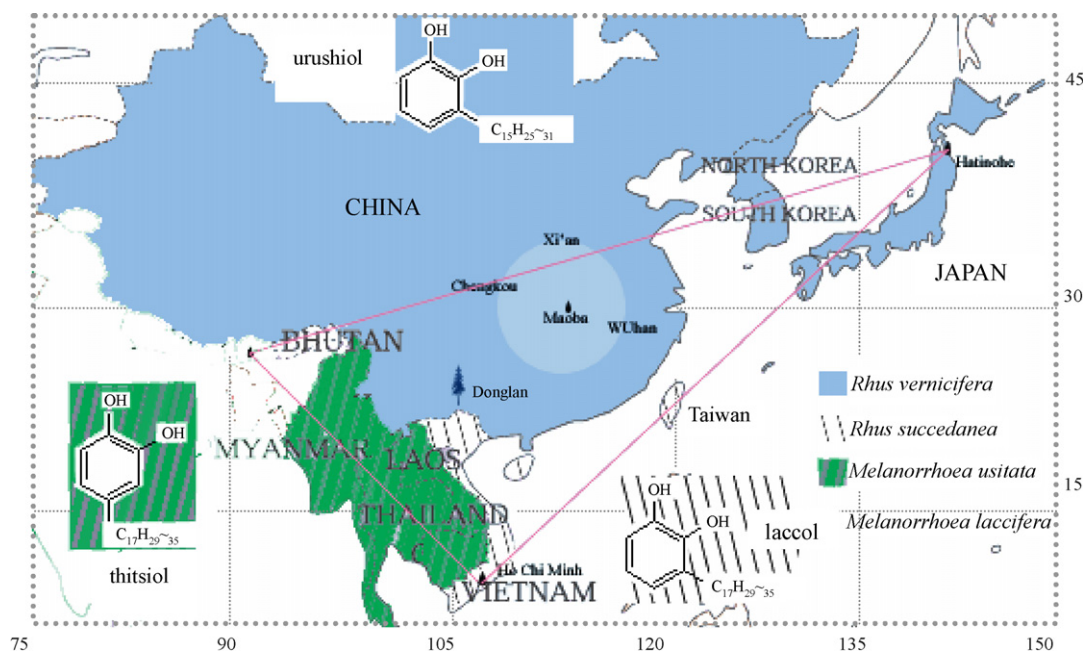


Fig. 1. Dominating distribution of lacquer trees in the world. Urushiol from *Rhus vernicifera*, laccol from *Rhus succedanea*, and thitsiol from *Melanorrhoea usitata* and *Melanorrhoea laccifera* were the main components of the sap collected from the trees mentioned above, respectively.

chromatography/mass spectrometry (Py-GC/MS) [16,17] was established to distinguish sap of Donglan (as shown in Fig. 1) trees from those of other species and compared with conventional methods in order to verify these results. The reason to choose Donglan lacquer trees was as follows: Donglan City is located in the southwest of Guangxi Province, China, and it is adjacent to northern Vietnam and similar to it in topography (karst landforms), weather, and climate (subtropical); it has not been determined yet if its lacquer trees are the *Rhus succedanea* species of northern Vietnam or the *Rhus vernicifera* species most frequent in China mainland, although it is more likely to be the latter based on the phytomorphology and geobotany [5,18]. Chengkou (shown in Fig. 1) lacquer is a typical Chinese lacquer [4,5,18,19] and was chosen for comparison as well as lacquers from Vietnam and Thailand.

2. Materials and methods

2.1. Lacquer samples and characteristics of appearance of the lacquer trees and leaves

The characteristics of the lacquer trees and leaves were directly obtained from visual observations according to the conventional morphology.

The original lacquer latex was collected from Donglan City, Guangxi Province, China (Fig. 1), in 2004. After filtration by the Chinese traditional method, this lacquer was treated according to the Kurome and Nayashi methods as follows: 20 g lacquer was stirred in an open vessel (bottom diameter, 80 mm; capacity, 100 ml) with cool air blowing at room temperature for 1 h until the water concentration was reduced to about 5% (determined by an AND MX-50 moisture analyzer, AND, Japan). Then it was coated on glass sheets at a thickness of 76 μm , and hardened in

a humidity-controlled chamber with 70–90% relative humidity at 20–40 °C. After 7 days, the lacquer-coated glass sheets were removed from the chamber and stored at room temperature for about 2 years [20,21]. Other raw lacquer samples were purchased from Tohtyuu Urushi-Ya in Osaka, Japan and were treated with the same methods described above.

2.2. Measurement of drying, IR, and NMR

The drying process of lacquer can be divided into three stages: dust-free dry (DF), touch-free dry (TF), and hardened dry (HD), using the automatic drying time recorder (RC painting time of drying auto-recorder, TaiYu Co. Ltd., Osaka, Japan) [22]. The pencil hardness (PH) standard consisting of letters and numbers is determined according to the current national standard of GB/T6739-1996: H and B designated the hardness and softness of the tested films, respectively; a higher number with H or B expresses the greater hardness or softness of the tested films, respectively; F and HB mean medium hardness films, while F is a slightly harder film than HB.

Infrared spectra (IR) were taken on a JASCO FTIR 460+ spectrometer in dry air at 25 °C. It is possible that the moisture in the lacquer sap would melt a KBr or NaCl disk so a polyvinylidene chloride (PVD) film (Saran Wrap, Asahi Kasei, Japan) was used to form the IR sample. The lacquer was thinly coated with a spatula on the PVD film, and the same kind of PVD film used applied as a blank. Spectra of samples were acquired at 2 cm^{-1} resolution ranged from 500 cm^{-1} to 4000 cm^{-1} under the same scanning and temperature conditions.

^1H NMR spectra were recorded on a 500 MHz JEOL JNM α -500 spectrometer. Samples are dissolved in DMSO- d_6 or CDCl_3 with chemical shifts referenced from tetramethylsilane (TMS).

Table 1
Morphology of lacquer trees from various countries or regions

| Origin | Scientific name | Shape of trees | Upper surface of leaves | Low surface of leaves | Carp |
|---|--------------------------------------|----------------------|--|---|----------------------------------|
| China, Japan, Korea ^a | <i>Rhus vernicifera</i> | Deciduous small tree | Leafstalks, rachises with capill | Stalks with thickening fur | Without capill on its surface |
| China, Japan, Korea ^b | <i>Rhus trichocarpa</i> Mig | Deciduous small tree | Leafstalks, petiolule with thickening capill | Stalks with thickening fur | With seta on its surface |
| Vietnam, Taiwan and Donglan region ^c | <i>Rhus succedanca</i> | Deciduous small tree | Without capill overall | Displayed white powder color | Without capill on its surface |
| China, Japan, Korea | <i>Rhus sylvestris</i> sieb | Deciduous small tree | Rachises with thickening capill | All of capill in irregular stocking | Without capill on its surface |
| China, Japan, Korea | <i>Rhus javanica</i> L. | Deciduous small tree | With sawtooth, rachis having wings | / | With thickening fuzz |
| China, Japan, Korea ^d | <i>Semecarpus vernicifera</i> Hayata | Deciduous small tree | Without capill | Base of lateral lobe with thickening brown capill | Short seta in irregular stocking |

^a Odd pinnately compound leaves with 4–7 pairs leaflets, sharp head.

^b Leaves of the younger with serrated edges.

^c Odd pinnately compound leaves with 4–8 pairs leaflets, sharp head.

^d Edges of small sapling with rough serration, changed red or yellow in fall.

2.3. GC–MS measurements

GC–MS was carried out with a HPGCD Plus GC instrument equipped with an HP 5 column (cross-linked 5% diphenyl and 95% dimethylpolysiloxane; 0.25 mm i.d. × 30 m, film thickness 0.25 μm). The temperature of the GC oven was increased at a constant rate of 20 °C/min from 40 °C to 280 °C, and then maintained for 10 min. The constant pressure of helium was 0.3 psi, and the split rate was 50:1. The samples in acetone solution were injected manually at 200 °C. The signals were detected by a FID at 240 °C.

2.4. Py-GC/MS measurements

The pyrolyzer used was a double-shot Py-2010D (Frontier, Japan) vertical microfurnace-type instrument along with

an Agilent 6890 (HP Agilent, USA) gas chromatograph and a JMS-AMSUN (JEOL, Japan) mass spectrometer. HPG5972A and HPG1701AJ models were used for the mass analysis and data processing, respectively. Stainless steel capillary columns (0.25 mm i.d. × 30 m) coated with a layer of 0.25 μm Ultra Alloy Py-1 and Py-2 (dimethylpolysiloxane 100%) film are used for separation. The 50 ml/min He gas flow at the pyrolyzer was reduced to 1 ml/min at the capillary column by a splitter. A 0.5 mg sample was put in a platinum cup, which was then placed on the top of the pyrolyzer at near ambient temperature. After the sample was placed in the furnace at 500 °C, the temperature of the GC oven was increased at a constant rate of 20 °C/min from 40 °C to 280 °C, then kept for 10 min. All pyrolysis products were analyzed by mass spectrometry. The mass spectrometer ionization energy (EI-MS) was 70 eV.

Table 2
Drying properties and hardness of lacquers under various drying conditions

| Source | Drying conditions | | Drying properties and hardness | | | | | | | |
|--------------------------|-------------------|--------|--------------------------------|-----|-----|-----|-----|-----|-------|--------|
| | Temperature (°C) | RH (%) | 1 h | 2 h | 3 h | 4 h | 5 h | 6 h | 1 day | 2 days |
| Thai raw lacquer | 20 | 90 | ND | ND | DF | DF | DF | DF | TF | HD |
| | 30 | 80 | ND | ND | ND | DF | DF | DF | HD | 6B |
| | 30 | 90 | ND | ND | DF | DF | TF | TF | HD | 6B |
| China raw lacquer | 20 | 90 | ND | TF | TF | TF | HD | HD | B | HB |
| | 30 | 80 | DF | TF | HD | 6B | 5B | 4B | F | F |
| | 30 | 90 | DF | HD | HD | HD | HD | HD | 3B | B |
| Vietnam raw lacquer | 20 | 90 | DF | TF | HD | HD | HD | HD | 5B | 4B |
| | 30 | 80 | ND | DF | DF | DF | TF | HD | HD | F |
| | 30 | 90 | DF | HD | HD | HD | HD | 6B | 6B | 6B |
| Donglan lacquer (summer) | 20 | 90 | ND | ND | DF | DF | DF | DF | HD | HD |
| | 30 | 80 | ND | TF | HD | HD | HD | 6B | 5B | B |
| | 30 | 90 | ND | TF | TF | TF | TF | HD | HD | F |
| Donglan lacquer (winter) | 20 | 90 | ND | ND | DF | DF | DF | DF | TF | TF |
| | 30 | 80 | ND | ND | DF | DF | DF | TF | TF | HD |
| | 30 | 90 | ND | DF | TF | TF | TF | TF | HD | HD |

Notes: ND, none dry; DF, dust free dry; TF, touch free dry; HD, harden dry; hardness: H > F > HB > B.

3. Results and discussion

3.1. Appearance of lacquer trees and leaves

Compared with the appearance of lacquer trees from various countries and regions, Table 1 shows clearly that Donglan lacquer trees have remarkable similarity to those of Vietnam. However, although related to bio-geographic region, they were historically regarded as Chinese lacquer trees. Both trees are small deciduous trees with odd pinnate compound leaves with 4–8 pairs of leaflets, and both upper and lower surfaces of the leaves are markedly different from those of Chinese mainland's *Rhus vernicifera* (Table 1), and so the historic classification of Donglan lacquer trees might be roughly based on speculation of geobotany or assumed as a matter of course due to the reasons of the traffic inconvenience or big financing consumption, etc.

3.2. Drying properties of lacquers from various countries or regions

It has been shown that the drying time of lacquers is related to temperature and humidity [5, 18]. Table 2 summarizes the test results. It was difficult to dry all these lacquers at room temperature under a 70% relative humidity (RH) (data not shown). Raw lacquer of China produced the best results. The Thai lacquer was the worst in the experimental conditions. The Donglan lacquer, however, was comparable to that of China, especially in the first step of harden drying (HD), which is very important in lacquer chemistry. The Vietnam lacquer was in between. It is clear that a temperature of 25–30 °C and ~80% RH are the most important parameters in the drying of all samples, except the Vietnamese lacquer sample, the drying of which seemed to depend on the RH and was strictly limited to 90%. It is interesting to note that Donglan lacquer dried more quickly in summer than in winter. The drying properties are related to enzymatic activity [5, 18, 23, 24], and a low temperature and short time could be directly proportional to it. It was confirmed that the enzyme activities were consistent with the drying rate of these lacquers (data not shown). Therefore, to a certain extent, adjustment of

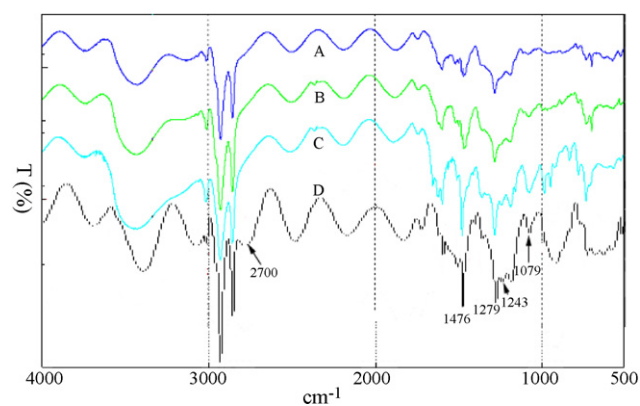


Fig. 2. IR of liquid lacquer from various countries and regions. From A to D were lacquer liquid of Thai, Chengkou region of China, Vietnam and origin Donglan region of China lacquer liquid, respectively.

the RH and temperature was able to produce optimal conditions for the enzyme activity.

3.3. Spectra and chromatographic analysis of lacquer liquids

3.3.1. FT-IR

The IR spectra of purified liquid lacquers from various countries and regions are shown in Fig. 2. Absorption due to the ethers of quinon-olefin and dibenzofuran appeared around 1470 cm^{-1} and 1080 cm^{-1} , respectively [23, 25]. The absorption at 1243 cm^{-1} was due to the stretching of the C–O–C group and deformation of the –OH group [26]. The peak at 2700 cm^{-1} was

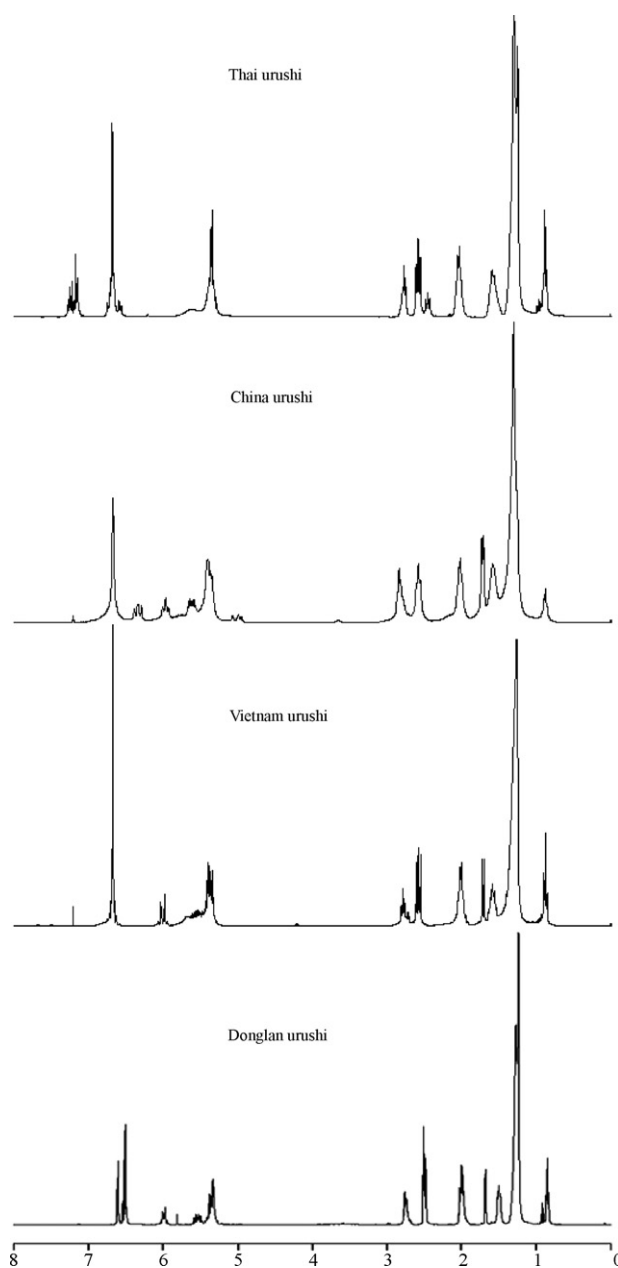


Fig. 3. ^1H NMR of liquid lacquers from various countries and regions. The three above, Thai, Chengkou, and Vietnamese liquid lacquers were detected in CDCl_3 ; the one below, Donglan liquid lacquer, was measured in DMSO-d_6 .

Table 3
Structure information of peaks in Fig. 4A

| Number | Structures |
|--------|----------------------------------|
| C5 | Pentane |
| C6 | 1-Hexene |
| C7 | Heptane |
| C8 | 1-Octene and/or octane |
| C9 | 1-Nonene and/or nonane |
| C10 | 1-Decene and/or decane |
| C11 | 1-Undecene and/or undecane |
| C12 | 1-Dodecene and/or dodecane |
| C13 | 1-Tridecene and/or tridecane |
| C14 | 1-Tetradecene and/or tetradecane |
| C15 | 1-Pentadecene and/or pentadecane |
| C16 | 1-Cetene and/or cetane |
| C17 | 1-Heptadecene and/or heptadecane |

due to the conjugated structure of triene [5] that was present only in lacquer of the Donglan region of China. In spite of that, it can be seen that the IR spectra of these lacquers were very similar. This can be attributed to the fact that the functional groups of these lacquers are similar in mass and the minor differences are not reflected by IR spectra.

3.3.2. NMR

Fig. 3 shows the ^1H NMR spectra of liquid lacquers from various countries and regions. It is clear that the Donglan lacquer has the same NMR properties as the others except that of Thailand lacquer. The peaks at 0.86–0.94 ppm and 1.2–1.4 ppm were the end methyl and the methylenes of the side chain, respectively. The peaks at around 1.75 ppm, 2.1 ppm and 2.8 ppm represented the end methyl, the methylene adjacent to the olefinic bond, and the methylene between the olefinic bonds ($-\text{C}=\text{C}-\text{CH}_3$, $-\text{C}=\text{C}-\text{CH}_2-$, and $-\text{C}=\text{C}-\text{CH}_2-\text{C}=\text{C}-$, respectively). The peak at 2.6 ppm was assigned to the methylene linked with the phenyl ring. The peaks around 4.9–6.1 ppm were due to the groups of olefinic bonds, and the peak at about 6.7 ppm represented the hydrogens of the benzene ring. The peaks at 7.17–7.27 ppm

Table 4
Structure information of peaks in Fig. 4B

| Number | Structures | Number | Structures |
|--------|---|--------|---|
| 1 | 2-Methylphenol | 1' | 3-Methylphenol |
| 2 | 2-Ethenylphenol and 2-ethylphenol | 2' | 3-Ethenylphenol and 3-ethylphenol |
| 3 | 2-Propenylphenol and 2-propylphenol | 3' | 3-Propenylphenol and 3-propylphenol |
| 4 | 2-Butenylphenol and 2-butylphenol | 4' | 3-Butenylphenol and 3-butylphenol |
| 5 | 2-Pentenylphenol and 2-pentylphenol | 5' | 3-Pentenylphenol and 3-pentylphenol |
| 6 | 2-Hexenylphenol and 2-hexylphenol | 6' | 3-Hexenylphenol and 3-hexylphenol |
| 7 | 2-Heptenylphenol and 2-heptylphenol | 7' | 3-Heptenylphenol and 3-heptylphenol |
| 8 | 2-Octenylphenol and 2-octylphenol | 8' | 3-Octenylphenol and 3-octylphenol |
| 9 | 2-Nonenylphenol and 2-nonylphenol | 9' | 3-Nonenylphenol and 3-nonylphenol |
| 10 | 2-Decenylphenol and 2-decylphenol | 10' | 3-Decenylphenol and 3-decylphenol |
| 11 | 2-Undecenylphenol and 2-undecylphenol | 11' | 3-Undecenylphenol and 3-undecylphenol |
| 12 | 2-Dodecenylphenol and 2-dodecylphenol | 12' | 3-Dodecenylphenol and 3-dodecylphenol |
| 13 | 2-Tridecenylphenol and 2-tridecylphenol | 13' | 3-Tridecenylphenol and 3-tridecylphenol |
| 14 | 2-Tetradecenylphenol and tetradecylphenol | 14' | 3-Tetradecenylphenol and tetradecylphenol |
| 15 | 2-Pentadecenylphenol and 2-pentadecylphenol | 15' | 3-Pentadecenylphenol and 3-pentadecylphenol |
| 16 | 2-Hexadecenylphenol and 2-hexadecylphenol | 16' | 3-Hexadecenylphenol and 3-hexadecylphenol |
| 17 | 2-Heptadecenylphenol and 2-heptadecylphenol | 17' | 3-Heptadecenylphenol and 3-heptadecylphenol |

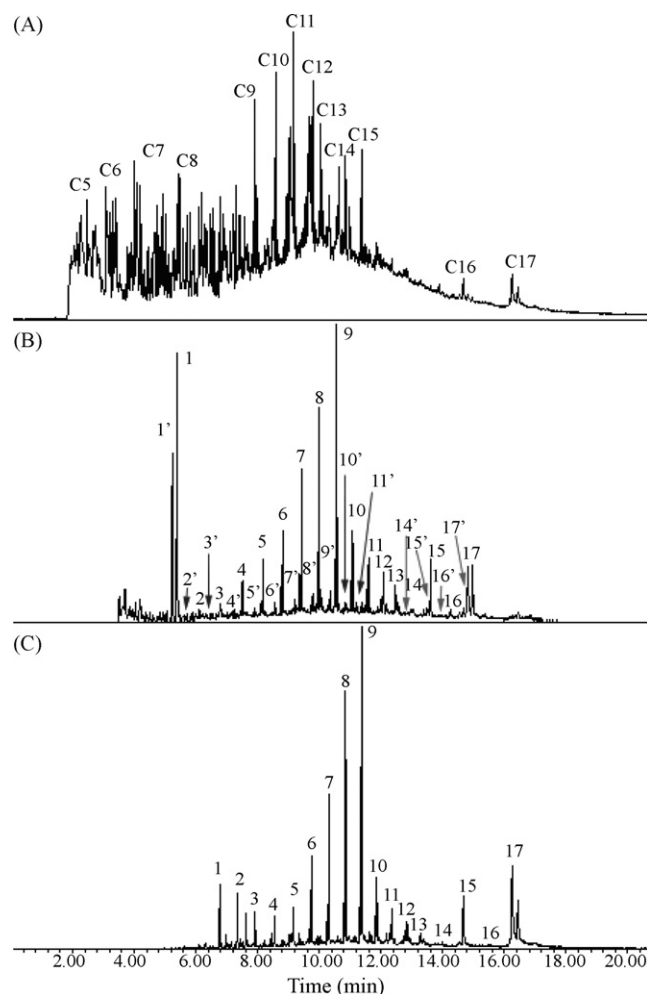
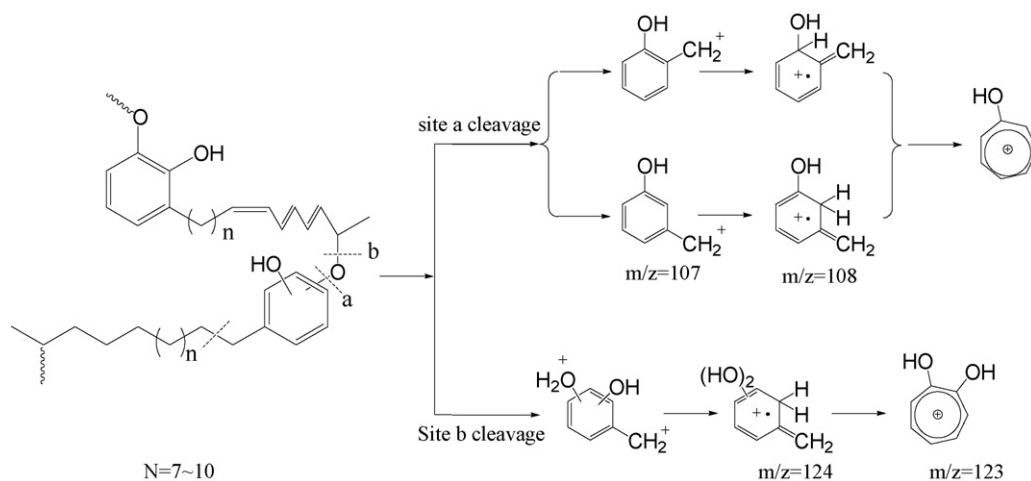


Fig. 4. TIC and mass chromatogram of Donglan lacquer film obtained by pyrolysis at 500 °C. (A) TIC, (B) mass chromatogram (m/z 108), (C) mass chromatogram (m/z 123).



Scheme 1. Postulated mechanism of formation of m/z 108 and m/z 123 by thermal decomposition of lacquer film.

of Thailand lacquer were the typical proton signals of the end phenyl of the alkyl side chains [4].

3.3.3. GC-MS and Py-GC/MS

The concentrations of saturated, monoene, diene, and triene of the liquid lacquers were different in summer and winter. This situation could be very common in natural products. Du et al. [27] reported that the concentration of urushiol in the sap of *Rhus vernicifera* was different in different seasons. It should be noted that new components, peaks at 9.753 min (Mw 416) and 15.349 min (Mw 402), were found in our experiments compared with that reported before [28,29], and the latter (Mw 402) was also found in the sap of *Rhus succedanea* (unpublished data). The peak at 9.753 min and 15.349 min, in some certain, these two new parts with phenylalkyl groups were similar to that from *Melanorrhoea usitate* [30].

The Donglan lacquer film was pyrolyzed at 500 °C. The total ion chromatogram (TIC) and the mass chromatogram are shown in Fig. 4, and the mass spectrometer analysis of the peaks

showed that the major components are alkanes, alkenes, alkyl and alkenyl-phenols. The detailed results were summarized in Tables 3–5.

It has been suggested that the m/z 123/124 and 107/108 specific ions were produced through McLafferty rearrangement when the film was thermally decomposed, as shown by Scheme 1 [31,32]. Because the intermediate heptatrienyl cation is the most stable and the trienyl urushiol/laccol has the highest potential for C–C and C–O coupling reactions [27,33], the trienyl part of lacquer was chosen as a model which is consistent with the main part in the lacquer sap (about 40% of the total in summer). This could explain why the lacquer dried faster in summer than in winter (which only contained about 3% in Donglan lacquer). In fact, a lacquer sap that contained a higher concentration of the triolefin gave a good performance as a coating material.

Py-GC/MS has been proven to be an effective method to analyze lacquer film [16,34]. Peak pairs of alkenylphenols and alkylphenols (larger peaks on the right of each alkenylphenol in Fig. 4, middle) were detected in the mass chromatograms (m/z 108) of the Donglan lacquer films that were similar to our previous studies [16,19]. The longest side chains were heptadecylphenols and/or heptadecenylphenols, which were the same as laccol from *Rhus succedanea* [34]. Peak pairs (Fig. 4, down) could also be detected in the mass chromatograms (m/z 123) and were consistent with mass chromatograms at m/z 108 (Fig. 4, Middle) except for the larger abundance of both and the greater overlap than that of the m/z 108, they were assigned the same number.

4. Conclusions

In conclusion, through the analysis of conventional physical morphology, drying properties, and especially results obtained by chromatography and spectroscopy, the concentrations and components of Donglan lacquer could place it in the category of Vietnam laccol, and its tree species could be totally attributed to *Rhus succedanea* of the Vietnam and Taiwan regions although some differences, instead of the species of the Chinese mainland, *Rhus vernicifera*, as was formerly thought [5]. According to the

Table 5
Structure information of peaks in Fig. 4C

| Number | Structures |
|--------|---|
| 1 | 3-Methylbenzene-1,2-diol |
| 2 | 3-Ethenylbenzene-1,2-diol and 3-ethylbenzene-1,2-diol |
| 3 | 3-Propenylbenzene-1,2-diol and 3-propylbenzene-1,2-diol |
| 4 | 3-Butenylbenzene-1,2-diol and 3-butylbenzene-1,2-diol |
| 5 | 3-Pentenylbenzene-1,2-diol and 3-pentylbenzene-1,2-diol |
| 6 | 3-Hexenylbenzene-1,2-diol and 3-hexylbenzene-1,2-diol |
| 7 | 3-Heptenylbenzene-1,2-diol and 3-heptylbenzene-1,2-diol |
| 8 | 3-Octenylbenzene-1,2-diol and 3-octylbenzene-1,2-diol |
| 9 | 3-Nonenylbenzene-1,2-diol and 3-nonylbenzene-1,2-diol |
| 10 | 3-Decenylbenzene-1,2-diol and 3-decylbenzene-1,2-diol |
| 11 | 3-Undecenylbenzene-1,2-diol and 3-undecylbenzene-1,2-diol |
| 12 | 3-Dodecenylbenzene-1,2-diol and 3-dodecylbenzene-1,2-diol |
| 13 | 3-Tridecenylbenzene-1,2-diol and 3-tridecylbenzene-1,2-diol |
| 14 | 3-Tetradecenylbenzene-1,2-diol and tetradecylbenzene-1,2-diol |
| 15 | 3-Pentadecenylbenzene-1,2-diol and 3-pentadecylbenzene-1,2-diol |
| 16 | 3-Hexadecenylbenzene-1,2-diol and 3-hexadecylbenzene-1,2-diol |
| 17 | 3-Heptadecenylbenzene-1,2-diol and 3-heptadecylbenzene-1,2-diol |

above analysis, when the basic information about a specimen is known, the pyrolysis products clearly show a good correspondence to the components of lacquer film and can be used to distinguish between species, and a new investigation on lacquer trees may be needed and urgent. It can be anticipated that, due to its ease of control, speed in analysis, and good reappearance, the Py-GC/MS method can be applied not only in lacquer film, organic coatings [16], and materials that cannot be dissolved in solvents, but also can be used to identify the species of trees, especially those with similar properties, as a new, replenishing and/or alternative method to identify and characterize plants.

Acknowledgements

The Authors thank Miss Ting Liu, School of Law, Wuhan University, for good ideas and useful help. This work was partly supported by the Research Project Grant A by the Institute of Science & Technology, Meiji University, and the National Natural Science Foundation of China (no. 30170757).

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