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Evaluation of Synthesized Lacquer Films Using Pyrolysis-Gas Chromatography/Mass Spectrometry

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The trienyl urushiols, which are major urushiol components, were synthesized via the Wittig reaction. The synthesized urushiols were then polymerized into synthesized lacquer films by laccase-catalyzed oxidation. The resulting lacquer films were characterized using Pyrolysis-GC/MS, and the results were compared with that of a natural lacquer film in order to evaluate their polymerization mechanism and molecular structure. Based on these results, it was found that natural lacquer films are terminated with the monoenyl and saturated urushiol, and that the polymerization of synthesized urushiol proceeds through the laccase-catalyzed nucleus-side chain C–O coupling and autoxidative side chain-side chain C–C coupling, like the natural lacquer film.

In a previous paper, we reported on the synthesis of 3-[(8*Z*,11*E*,13*Z*)-8,11,13-pentadecatrienyl]catechol (**1**), 3-[(8*Z*,11*E*,13*E*)-8,11,13-pentadecatrienyl]catechol (**2**), and 3-[(8*Z*,11*E*)-8,11,14-pentadecatrienyl]catechol (**3**), which are major urushiol components.[1] The stereostructures of compounds **1—3** are shown in Fig. 1. In general, a natural lacquer film is obtained by the laccase-catalyzed oxidative polymerization of urushiol.[2—5] Accordingly, these synthesized urushiol analogs and urushiol components are expected to undergo polymerization to produce lacquer films similar to natural lacquer films by laccase-catalyzed oxidation.

We previously reported that a pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) technique is effective for the analysis of lacquer films, because it can distinguish the pyrolysis products of glycoprotein and monomeric phenol derivatives as well as those of the polymeric phenol derivatives.[6,7] Because urushi films are insoluble in solvents, only a few analytical techniques are now available, such as solid NMR,[8] FT-IR,[9,10] and XPS. But Py-GC/MS analysis is not only a fast technique, but also requires only small amounts of sample and no sample work-up.

There is no thermal degration data of a single urushiol component's film using Py-GC/MS. Thus, in this paper three synthesized urushiols **1—3** were polymerized into three synthesized lacquer films by laccase-catalyzed oxidation. The resulting synthesized lacquer films were then characterized using Py-GC/MS. In addition, the results were compared with that of a natural lacquer film in order to evaluate their polymerization mechanism and molecular structure, as well as the difference between natural and synyhesized lacquer films.

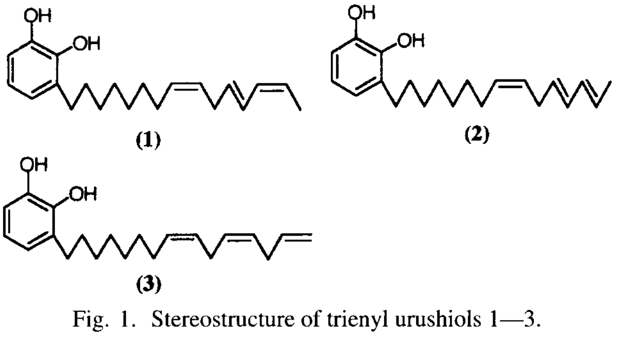


Fig. 1. Stereostructure of trienyl urushiols 1—3.

Experimental

Synthesis of 3-[(8Z,11*E*,13Z)-8,11,13-pentadecatrienyl]catechol (1). This compound, the major component of urushiol, was synthesized in good yield via the Wittig reaction from a ylide derived from (3*E*,5*Z*)-3,5-heptadienyltriphenylphosphonium iodide with 3-(8-oxo-1-octyl)catechol diacetate, using a stepwise procedure based on repeated protection and deprotection of the hydroxy group of catechol.[11]

Synthesis of 3-[(8Z,11*E*,13*E*)-8,11,13-Pentadecatrienyl]catechol (2) and 3-[(8Z,11*E*)-8,11,14-pentadecatrienyl]catechol (3). Trienyl urushiols **2** and **3** were synthesized in a similar fashion using ylides derived from (3*E*,5*E*)-3,5-heptadienyltriphenylphosphonium iodide and 3*E*-3,5-heptadienyltriphenylphosphonium iodide, respectively.

Preparation of Synthetic Lacquer Film. Compound **1** (50 mg) was added to a 10 mg water-isopropyl alcohol mixture (1 : 1, v/v) containing acetone powder (10 mg), which was separated as an acetone-insoluble material from the sap of a lacquer tree containing non-oily parts, i.e., plant gum, glycoprotein and laccase enzyme. The resulting mixture was stirred for 15 min. The reaction mixture had a viscosity suitable for coating, and was coated on a glass plate, which was dried in a humidity-controlled chamber with an RH of 80% at 25—30 °C for 10 h. The mixture was polymerized into a lacquer film, which was similar to natural lacquer films. The film was then removed from the chamber and place in air for 3 years. Compounds **2** and **3** were treated in the same manner to obtain the corresponding lacquer films. The films from trienyl urushiols **1**, **2**, and **3** are called synthesized lacquer films A, B, and C, respectively.

Natural Lacquer Film. Sap exuded from the *Rhus vernicifera* (Japanese lacquer tree, urushiol composition: saturated urushiol 5%, monoenyl urushiol 18%, dienyl urushiol 12%, trienylurushiol 65%) was successively treated according to the traditional Kurome and Nayashi procedures[12] and coated on a glass plate, then hardened in a humidity-controlled chamber with an RH of 80% at 25—30 °C for 10 h. It was then removed from the chamber and placed in air for 3 years.

Py-GC/MS Conditions. Py-GC/MS analyses were carried out using a PY-2010D (Frontier Lab, Japan) vertical microfurnace-type pyrolyzer, an HP6890 (Hewlett-Packard, Ltd.) gas chromatograph, and a HPG5972A (Hewlett-Packard, Ltd.) mass spectrometer. A stainless-steel capillary column (0.25 mm i.d. ×30 m) coated with 0.25 μm of Ultra Alloy PY-1 (100% methylsilicone) was used for the separation. He carrier gas with a flow rate of 50 ml min^{-1} at the pyrolyzer was reduced to 1 mL min^{-1} at the capillary column using a splitter. The sample (1.0 mg) was placed in a platinum cup. The cup was placed on top of the pyrolyzer at near ambient temperature. The sample cup was introduced into the furnace at 500 °C, then gas chromatographed. The GC was programmed at a constant temperature increase of 20 °C min^{-1}, starting from 40 to 280 °C, and then held for 10 min. Most pyrolysis products were detected and identified by mass spectrometry (70 eV; EI-mode).

Results and Discussion

The three synthesized lacquer films and a natural lacquer film were pyrolyzed at 500 °C. The resulting pyrolysis products were then characterized by GC/MS analysis. Figure 2 shows the resulting TICs. A mass-spectrometer analysis of the TIC peaks showed that the major components are alkanes, alkenes, and alkylbenzenes. When urushi film was thermally

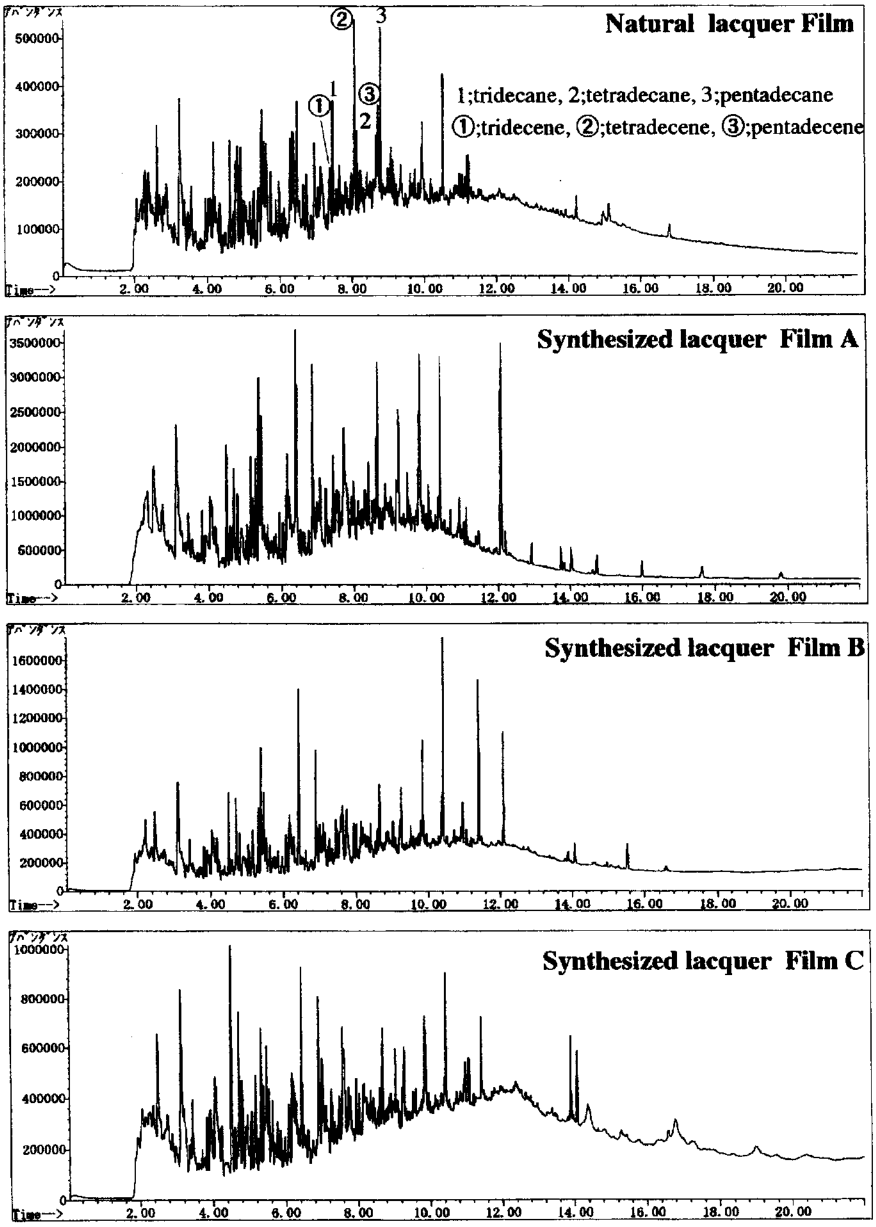
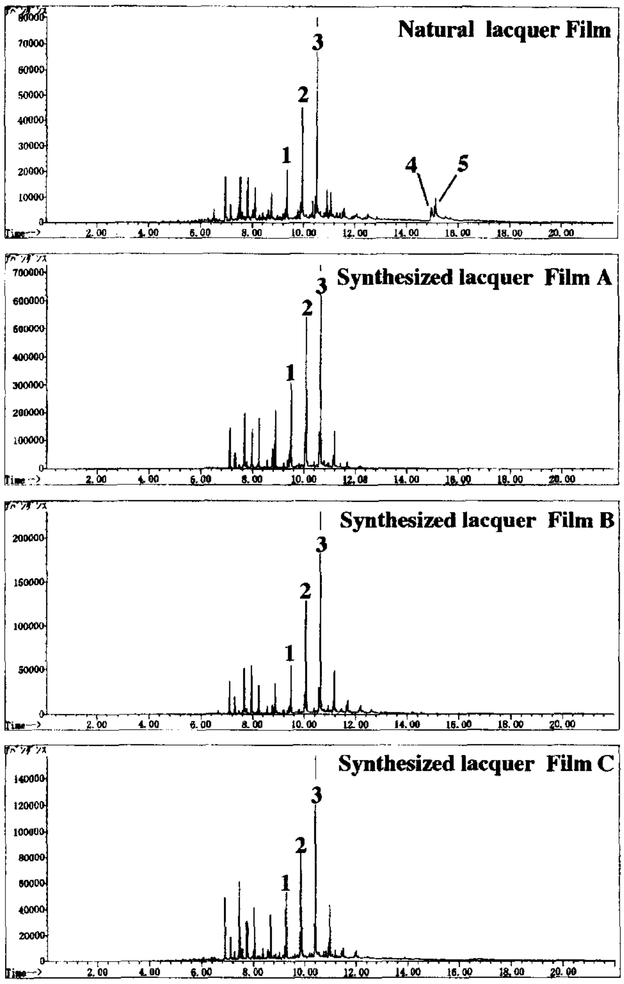


Fig. 2. Total ion chromatogram of the synthesized and natural lacquer films.

decomposed, it was guessed that the m/z 123 and 108 specific ion were produced as shown in Scheme 1.[13] Figure 3 shows the selective scanning of m/z 123 in these mass spectra. Peaks 1—3 in Fig. 3 were identified as 3-pentylcatechol (M^+, 180), 3-hexylcatechol (M^+, 194) and 3-heptylcatechol (M^+, 208), respectivery, important pyrolysis products of urushiol. In addition, the selective scanning of m/z 123 from natural lacquer film contained peaks 4 and 5. These peaks were identified as those of the natural urushiol components, 3-pentadecenylcatechol (M^+, 318) and 3-pentadecylcatechol (M^+, 320), based



1:3-pentylcatechol (MW 180), 2:3-hexylcatechol (MW 194), 3:3-heptylcatechol (MW 208), 4:3-pentadecenylcatechol (MW 318), 5:3-pentadecylcatechol (MW 320).

Fig. 3. Selective plotting of ion species m/z 123 in the spectra obtained from TIC of both three synthesized and natural lacquer films.

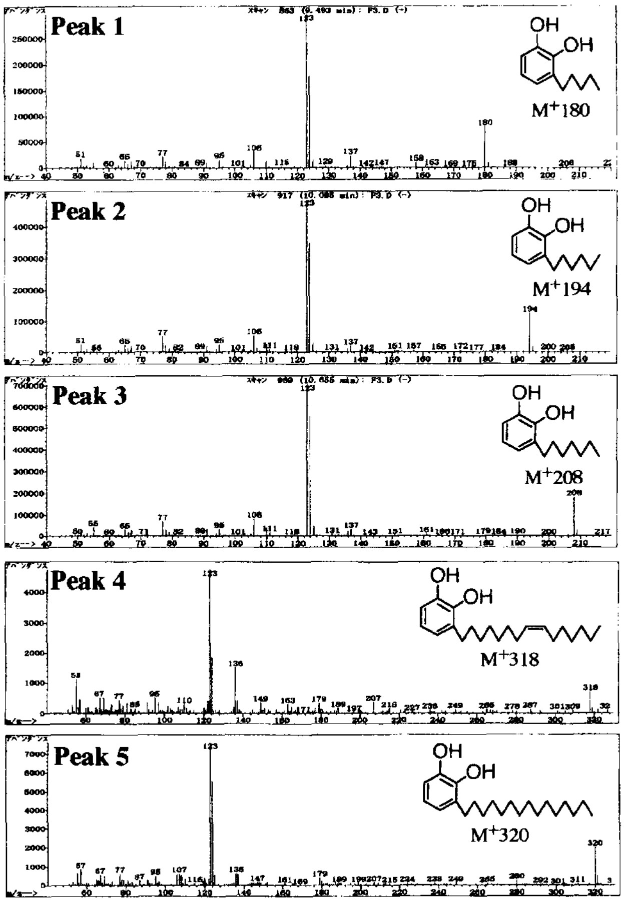
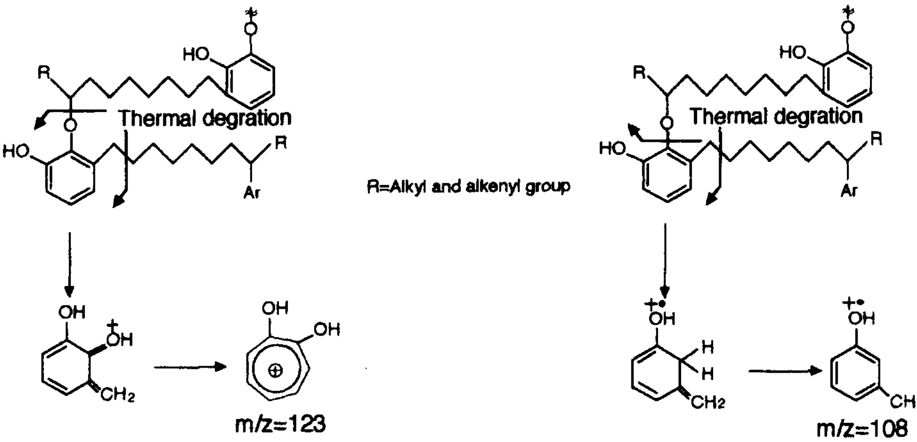
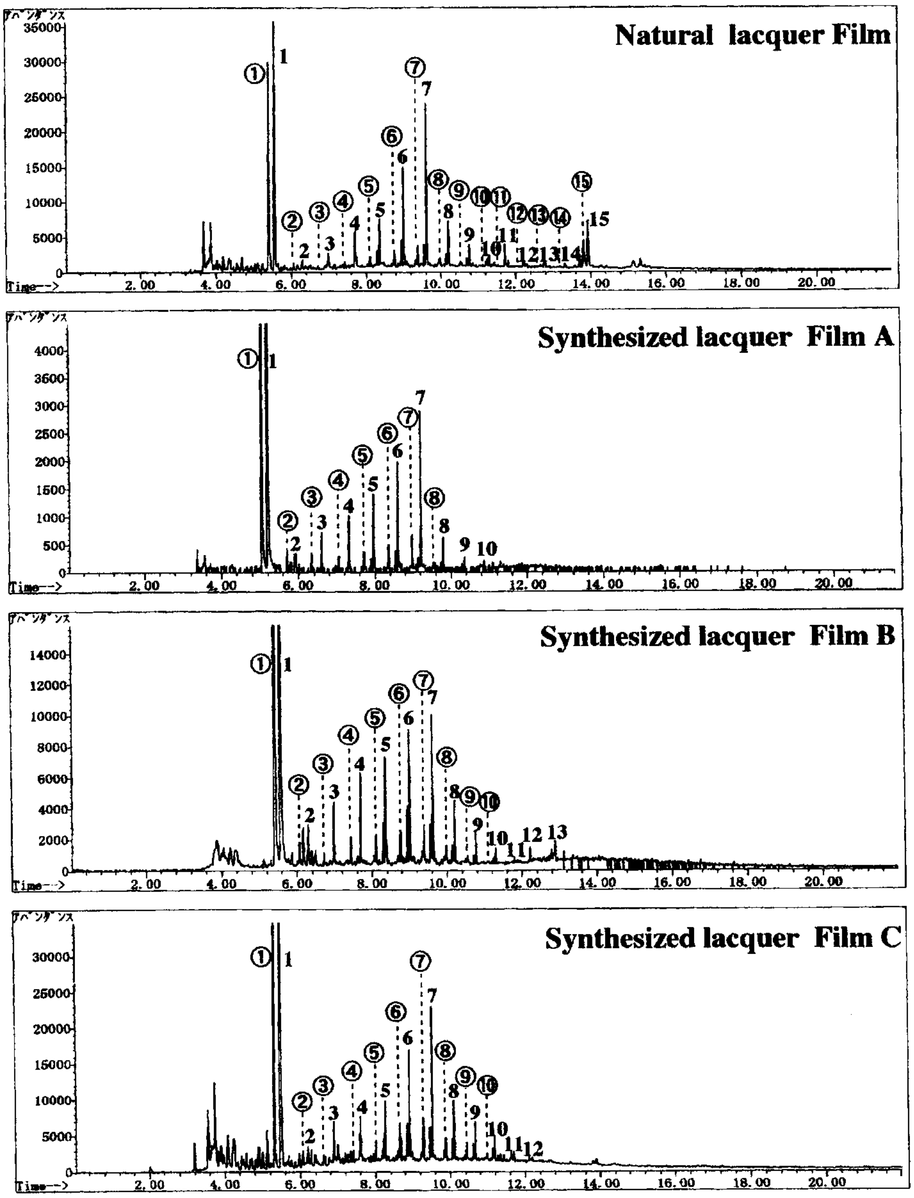


Fig. 4. Mass spectra of alkylcatechols from synthesized lacquer film (A) (Peaks 1—3) and natural lacquer film (Peaks 4 and 5).



Scheme 1. Formation of m/z 123 and 108 ion species by degration of urushi films.

on their mass spectra. Figure 4 shows the mass spectra of compounds giving rise to peaks 1—5 in the selective plotting of *m*/*z* 123 ion species of the spectra from the pyrolysis of synthesized and natural lacquer film in Fig. 3. Thus, it is evident from Fig. 3 that saturated urushiol (peak 5) and monoenyl urushiol (peak 4) were detected from the pyrolysis of the natural lacquer film, but not from the pyrolysis of the synthesized lacquer films. These urushiols are likely produced as thermally decomposed fragments from the terminal alkyl- and alkenylcatechol side chains of the natural lacquer film. Therefore, the natural lacquer film has urushiol components with both saturated and monoenyl side chains,



①:2-methylphenol, ②:2-ethenylphenol, 2-ethylphenol, ③:2-propenylphenol, 2-propylphenol, ④:2-butenylphenol, 2-butylphenol, ⑤:2-pentenylphenol, 2-pentylphenol, ⑥:2-hexenylphenol, 2-hexylphenol, ⑦:2-heptenylphenol, 2-heptylphenol, ⑧:2-octenylphenol, 2-octylphenol, ⑨:2-nonenylphenol, 2-nonylphenol, ⑩:2-decenylphenol, 2-decylphenol, ⑪:2-undecenylphenol, 2-undecylphenol, ⑫:2-dodecenylphenol, 2-dodecylphenol, ⑬:2-tridecenylphenol, 2-tridecylphenol, ⑭:2-tetradecenylphenol, 2-tetradecylphenol, ⑮:2-penyadecenylphenol, 2-pentadecylphenol.
1:3-methylphenol, 2:3-ethenylphenol, 3-ethylphenol, 3:3-propenylphenol, 3-propylphenol, 4:3-butenylphenol, 3-butylphenol, 5:3-pentenylphenol, 3-pentylpheno, 6:3-hexenylphenol, 3-hexylphenol, 7:3-heptenylphenol, 3-heptylpheol, 8:3-octenylphenol, 3-octylphenol, 9:3-noneylphenol, 3-nonylphenol, 10:3-decenylphenol, 3-decylphenol, 11:3-undecenylphenol, 3-undecylphenol, 12:3-dodecenylphenol, 3-dodecylphenol, 13:3-tridecenylphenol, 3-tridecylphenol, 14:3-tetradecenylphenol, 3-tetradecylphenol, 15:3-pentadecenylphenol, 3-pentadecylphenol.

Fig. 5. Selective plotting of ion species *m*/*z* 108 in the spectra obtained from TIC of both three synthesized and natural lacquer films.

unlike the synthesized lacquer films.

Figure 5 shows the selective plotting of *m*/*z* 108 ion species in the spectra obtained from the TIC of both the three synthesized and natural lacquer films. Peak pairs of alkenylphenols (much smaller peaks on the left side) and alkylphenol (larger peaks on right side) were detected in both mass chromatograms, as shown in Fig. 5. The peak pairs of ①—⑮ were identified as 2-alkyl- and alkenyl phenols. On the other hand, the peak pairs of 1—15 were identified as 3-alkyl- and alkenyl phenols. Additionally, the alkenylphenols and alkylphenols having the longest side chain are shown to be a pentadecenylphenol and a pentadecylphenol in the mass chromatogram of the natural lacquer film. Figure 6 shows the mass spectra of peak pair 7 from the synthesized lacquer film. These alkenylphenols and alkylphenols are the pyrolysis products of the nucleus-side chain C–O coupling urushiol polymers, because it has been inferred that dimerization of urushiol proceeds through the laccase-catalyzed nucleus-side chain C–O coupling as well as the C–C coupling.[14] Furthermore, because the C–O coupling polymers should mainly terminate with alkyl- and monoenyl catechols, a pentadecenylphenol and a pentadecylphenol are formed from such terminal groups. On the other hand, a pentadecenylphenol and a pentadecylphenol were not detected in the mass chromatogram (m/z 108) of the synthesized lacquer films, because the synthesized urushiols were trienyl urushiols. Therefore, these lacquer films are not terminated with alkyl- and alkenylcatechols.

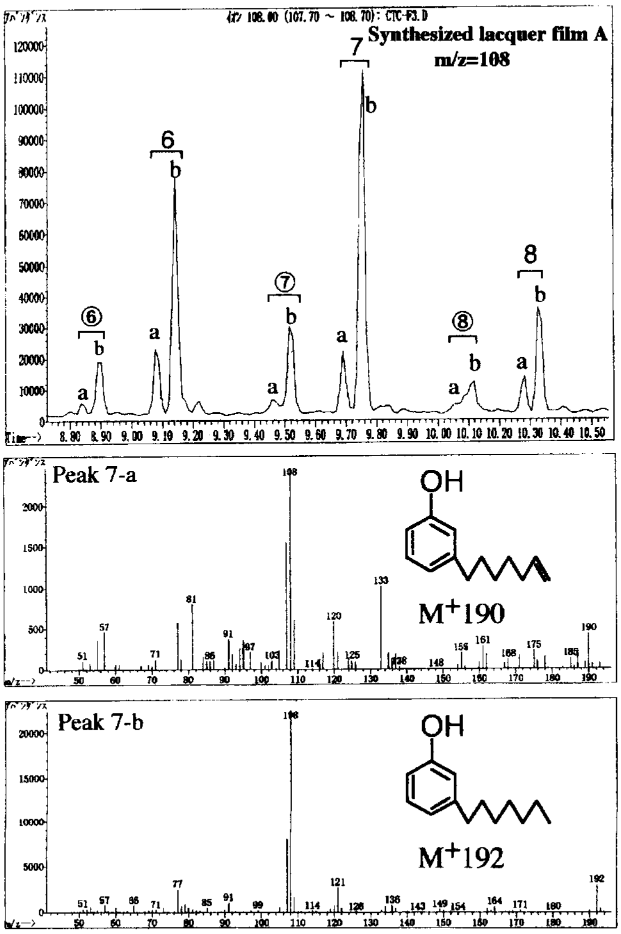


Fig. 6. Expanded ion chromatogram (*m*/*z* 108) and mass spectra of peaks 7-a and 7-b from synthesized lacquer film A.

The results discussed above indicate that polymerization of the synthesized urushiols proceeds through the laccase-catalyzed coupling of phenoxyl (PhO•) and allylic (–CH_2–CH=CH–CH-) radical species, as does the formation of natural lacquer film. Only one reference is that the natural lacquer film is mainly terminated with alkyl- and monoenyl catechol, unlike the synthesized lacquer films.

The peak intensity of the alkenes and alkanes was high enough to be detected in the TIC of the natural lacquer film, as shown in Fig. 2. On the other hand, the alkenes and alkanes were detected in the mass chromatograms (*m*/*z* 85, 83) of the three synthesized lacquer films, because the amounts of alkenes and alkanes produced from them were too small to be detected by TIC. These alkenes and alkanes are attributed to the side chains of urushiol, especially the terminal groups, which are alkylcatechol and monoenyl catechol. Therefore, these results suggest that the natural lacquer film is mainly terminated with the alkylcatechol and monoenyl catechol, but not the synthesized lacquer films.

In natural lacquer film, some alkanes and alkenes have carbon chains longer than the side chains of urushiol. Lacquer is reported to undergo autoxidation in air in addition to laccase-catalyzed oxidative coupling.[15] Moreover, dimers of urushiol with an oxidized side chain were identified in the laccase-catalyzed polymerization of natural lacquer.[16] In general, the oxidation mechanisms for the polymerization of unsaturated fatty acids may be applicable to the autoxidative coupling of the side chain of the urushiols. During the autoxidation of an unsaturated fatty acid, molecular oxygen may undergo a metal-complex catalyzed oxidation to produce reactive oxygen species, such as superoxide and peroxide as precursors.[17—19] These reactive oxygen species are likely to react with the double bonds of the fatty acid. However, only limited structural information is available on the secondary oxidation products of unsaturated fatty acid, such as linoleate and linolenate.[20] Similarly, the autoxidation of the unsaturated side chain of urushiol leads to the formation of hydroperoxides. These hydroperoxides participate in the cross-linking reaction of urushiol as well as in the autoxidative polymerization of unsaturated fatty acids.[21] Conceivably, dimeric products generated by the chain C–C coupling of the side chain-side in urushiol by autoxidation were cleaved by pyrolysis. This would lead to the forming of long-chain alkanes and alkenes. Further research is required to establish the reaction mechanism for the formation of the long-chain hydrocarbons is the pyrolysis of urushiol under an inert atmosphere.

It is observed that some alkanes and alkenes also have carbon chains longer than the side chain of the synthesized urushiol in the synthesized lacquer films. These must be the pyrolysis products of polymers of the synthesized urushiol with long side chain-side chains produced from the C–C coupling by autoxidation, because the synthesized urushiol has an unsaturated side chain, as does natural urushiol. These side chains lead to a cross-linking reaction of the synthesized urushiols.

As already discussed, the polymerization of synthesized urushiols proceeds through autoxidation involving side chain-side chain C–C coupling in addition to the laccase-catalyzed aryl-side chain coupling and phenoxyl-side chain coupling during the polymerization of natural urushiol.

Conclusion

The synthesized lacquer films, which were obtained by the laccase-catalyzed polymerization of the synthesized urushiols, **1**—**3**, were characterized using pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS). The results were compared with the characteristics of the natural lacquer film. The saturated and monoenyl urushiol components were detected in the natural lacquer film, but not in the synthesized lacquer films. However, alkylphenols, alkenylphenols, alkanes, and alkenes having longer carbon chains than the side chains of the synthesized urushiol were detected by Py-GC/MS analysis.

Based on these results, it is evident that a natural lacquer film has terminal moieties containing saturated and monoenyl urushiol components, but not the synthesized lacquer films. Moreover, polymerization of the synthesized urushiols proceeds through the laccase-catalyzed coupling between the aryl radical species and the C-centered radical species in the side chains, coupling between the phenoxyl radical species and the C-centered radical species in the side chain, and autoxidation involving double bonds in the side chains, as does a natural lacquer film. Because the structure and polymerization mechanism of the synthesized lacquer films are similar to those of a natural lacquer film, the synthesized lacquer films are tough and pretty enough to be used as a preservative surface coating material, like a natural lacquer film.

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