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Enzymatic Dehydrogenative Polymerization of Urushiols in Fresh Exudates from the Lacquer Tree, *Rhus vernicifera* DC

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Fresh exudates from the lacquer tree, *Rhus vernicifera* DC, were extracted with acetone and the solution was chromatographed to isolate monomer, dimer, trimer, and oligomer fractions of urushiols. Constituents of the monomeric and dimeric fractions were then identified by two-dimensional (2D) $^{1}H-^{13}C$ heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond coherence (HMBC) NMR spectroscopic techniques. The results showed that the monomeric fraction contained 3-[8′*Z*,11′*E*,13′*Z*-pentadecatrienyl]catechol (**1**), 3-[8′*Z*,11′*Z*,14′-pentadecatrienyl]catechol (**2**), and 3-pentadecanyl]catechol (**3**), which was verified by HPLC analysis. The dimeric fraction contained 8′-(3″,4″-dihydroxy-5″-alkenyl)phenyl-3-[9′*E*,11′*E*,13′*Z*-pentadecatrienyl]catechol (**4**), 14′-(3″,4″-dihydroxy-5″-alkenyl)phenyl-3-[8′*Z*,10′*E*,12′*E*-pentadecatrienyl]catechol (**5**), 2-hydroxyl-3- or -6-alkenylphenyl ethyl ether (**6**), 14′-(3″,4″-dihydroxy-2″-alkenyl)phenyl-3-[8′*Z*,10′*E*,12′*E*-pentadecatrienyl]catechol (**7**), 15′-(2″-hydroxy-3″- or -6″-alkenyl)phenyloxy-3-[8′*Z*,11′*Z*,13′*E*)-pentadecatrienyl]-catechol (**8**), 14′-(2″,3″-dihydroxy-4″-alkenyl)phenyl-3-[8′*Z*,10′*E*,12′*E*-pentadecantrienyl]catechol (**9**), 1,1′,2,2′-tetrahydroxy-6,6′-dialkenyl-4,3′-biphenyl (**10**), 1,1′,2,2′-tetrahydroxy-6,6′-dialkenyl-4,4′-biphenyl (**11**), 1,1′,2,2′-tetrahydroxy-6,6′-dialkenyl-5,4′-biphenyl (**12**), and 1,2,1′-trihydroxy-6,6′-dialkenyldibenzofuran (**13**) as constituents. In addition, dimeric ethers and peroxides, such as compounds **14** and **15**, were produced by autoxidation of monomeric urushiols in atmospheric air. The possible reaction mechanisms for the dehydrogenative polymerization of urushiols by *Rhus* laccase present in the fresh raw exudates under the atmospheric oxygen are discussed on the basis of structures identified. This is of primary importance because the use of the urushi exudates as coating materials does not involve organic solvents and is an environmentally friendly process.

KEYWORDS: Lacquer tree exudates; urushiols; laccase; autoxidation; single-electron-transferring oxidation; polymerization; 2D-NMR spectroscopy; reaction mechanisms

INTRODUCTION

When urushi exudates from lacquer trees are dried, they produce a tough and brilliant film and have been used as naturally occurring coating materials. Because of these characteristics, lacquerware has been an important folk art in Japan, China, and south-east Asia over the past 5000−7000 years (*1*−*5*). In addition, the use of urushi exudates as coating materials is an environmentally friendly process, since it does not involve organic solvents (*5*−*11*).

Lacquer trees belong to the genus *Rhus,* a member of the family Anacardiaceae that contains 73 genera worldwide with approximately 600 species, including mango (*Mangifera indica*) and cashew (*Anacardium occidentale*) (*3*). In general, there are eight species of representative of lacquer trees growing in east Asia, namely, *Rhus vernicifera* DC (Japan, China, Korea), *Rhus trichocarpa* Miq (China, Japan, Korea), *Rhus sylvestris* Sib (China, Japan, Korea), *Rhus anbiqua* L. (China, Japan, Korea), *Rhus javanica* L. (China, Japan, Korea), *Rhus succedanea* L. (China, Japan, Korea), *Rhus succedanea* L. var. Dumoutiere Kudo and Matura (Taiwan, Vietnam), and *Melanorrhoea usitata* wall (Thailand, Myanmar).

Urushi exudates are collected by artificially wounding a lacquer tree, such as *R. vernicifera* DC (Anacardiaceae), so that it secretes exudates that are called "urushi". An 8−13-year-old mature lacquer tree produced only about 200 g of fresh exudates per year by wounding the trunk of the tree in the middle of June to the end of October (*3*). It is well-established that the acetone-soluble part of the fresh exudates of *R. vernicifera* contained approximately 70% monomeric urushiols (*12*). The major constituents of the acetone-soluble fresh exudates of lacquer trees are different according to the region in which the lacquer trees are grown. For example, the fresh exudates of *R. vernicifera* grown in China, Japan, and Korea contain urushiols, while those of *R. succedanea* L. var. Dumoutiere

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Kudo and Matura grown in Taiwan and Vietnam contain laccols, and those of *M. usitata* grown in Thailand and Myanmar contains thitsisols (*2*−*5*).

When urushi exudates are dissolved in acetone, the resulting acetone-soluble part is the lipid fraction (acetone-soluble fraction) containing mostly urushiols and water, while the acetone-insoluble part is called the acetone powder. When acetone powder is dissolved in water, the resulting water-soluble part contains saccharides and copper glycoproteins, that is, enzymes including laccase, peroxidase, and stellacyanin. The water-insoluble part mostly consists of glycoproteins (*2*−*5*). Under oxygen present in atmospheric air, the monomeric urushiols undergo autoxidation and dehydrogenative polymerization by *Rhus* laccase present in the exudates during transportation from the plantation to the factory for processing. Therefore, it is of primary importance to elucidate the change in the constituents of the raw urushi exudates as well as the mechanisms of the polymerization.

In 1985, Oshima et al. (*13*) investigated the enzymatic oxidative coupling of urushiols in exudates of the lacquer tree. They showed that urushiols in the exudates underwent dehydrogenative coupling catalyzed by laccase under atmospheric oxygen, producing dimeric products. These products include coupling of phenoxyl radicals and monomeric urushiols with the conjugated systems in their side chains; coupling among phenyl radicals derived from phenoxyl radicals producing various biphenyl derivatives; and formation of dibenzofurans from oxidative dehydration of the resulting phenolic biphenyl derivatives when phenolic hydroxyl groups in the biphenyl derivatives are ortho to the biphenyl bridge. Three years later, Takeda et al. (*14*) used 4-*tert*-butyl-*o*-benzoquinone, 1,4-dipentadiene, methyl sorbate, methyl linoleate, and 3-[8′*Z*,11′*E*,-13′*Z*]-pentadecatrienyl]veratrol as model compounds to verify these conclusions. However, no structures for the constituents of raw urushi exudates or reaction mechanisms for polymerization of monomeric urushiols in the fresh exudates to produce dimeric, trimeric, and oligomeric products during transportation are firmly established.

The objective of the present studies was, therefore, to reinvestigate changes that occur in the constituents of the raw urushi exudates by modern techniques including changes in the molecular distribution pattern and advanced $^{1}H-^{1}H$ and $^{1}H-^{13}C$ correlation 2D-NMR spectroscopic techniques such as homonuclear shift correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond coherence (HMBC) sequences (*15*). These 2D-NMR spectroscopic techniques are widely used to elucidate structures of complex mixture of macromolecular compounds, such as polysaccharides (*16*) in lacquer, in addition to new natural products.

MATERIALS AND METHODS

Raw urushi exudates, produced from trees of *Rhus vernicifera* DC growing in Cheng-Kou in the Hubei province, China, were purchased from Tohityu-Ya, Osaka, Japan.

Gel-Permeation Chromatography. Gel-permeation chromatography was performed on a Tosho GPC system equipped with a system of three connecting 300 mm × 7.8 mm i.d. columns, TSK gels α-3000, α-4000, and α-M (Tosho, Tokyo, Japan), at 40 °C. A sample of raw urushi exudates (100 mg) was dissolved in 5 mL of DMF. After the resulting solution was filtered through a microdisk H-13-5 (Tosho, Tokyo, Japan), the sample solution was injected onto the column system and eluted with DMF solution containing 0.01 mol of LiBr/L in DMF at a flow rate of 0.8 mL/min. The eluent was monitored with an RI refractive index detector, model RI-8012 (Tosho, Tokyo, Japan). The relative mass was calculated from a calibration curve prepared from elution volumes of standard polystyrenes with molecular weights of 5.2×10^2, 2.63×10^3, and 1.81×10^4.

Separation of Monomers, Dimers, Oligomers, and Polymers from Raw Urushi Exudates. Raw urushi exudates were initially chromatographed by thin-layer chromatography (TLC), with silica gel as stationary phase and $CHCl_3$ as solvent as a preliminary test, and then were fractionated on a recycle HPLC LC-908 system (Japan Analytical Co. Ltd., Tokyo, Japan) equipped with an HPLC column [Tosoh, TSK-gel G1000H6, 7.6 mm (i.d.) × 300 mm × 2; exclusion limit M_r = 1000] and an RI and UV (254 nm) detector. The sample (5 mg) was dissolved in 2 mL of $CHCl_3$. The resulting solution was taken in a 5 mL syringe and injected into the column through a pretreatment PTFE cartridge containing an H-13-5 filter disc into the column, and then the column was eluted with $CHCl_3$ at a rate of 3.5 mL min^{-1}.

Infrared Spectroscopy. IR spectra were taken on a FT-IR460+ spectrometer (Jasco, Tokyo, Japan).

$^{1}H-^{13}C$ Correlation 2D-NMR Spectroscopy. NMR spectra were recorded on a JEOL model JNM-ECA500 (500 MHz) spectrometer with DMSO-d_6 containing 0.01% TMS as solvent. The system was controlled by an IBM RS/6000 43P model 150 host workstation and AIX V 4.3.2 (IBM UNIX) operating system. The data were processed by use of Delta V 4.2.3 (JEOL, Boston, MA) NMR software.

RESULTS AND DISCUSSION

Constitutions of Raw Urushi Exudates. In the lacquer tree, the lipid fraction usually contains almost 100% monomeric urushiols. However, when urushi exudates are collected in the plantation, filtrated to remove any solid materials, and then transported to processing, the amount of monomeric urushiols in the lipid fraction usually decreased to approximately 70% of the fraction because the exudates undergo oxidative polymerization catalyzed by *Rhus* laccase present in the exudates. In order to determine the degree of polymerization, the constituents of raw urushi exudates were quantified by gel-permeation chromatography (GPC). The resulting molecular distribution pattern showed that the peaks at the elution volumes of 50−60 mL, 42−47.2 mL, and 38.8−40.8 mL were determined to have average relative molecular mass (M_r) of approximately 300, 600, and 1000, corresponding to monomeric, dimeric, and trimeric fractions, respectively (**Figure 1**), according to standard polystyrene calibration curves. Raw urushi exudates were then dissolved in acetone. The resulting acetone-soluble part was chromatographed by HPLC to obtain three major fractions, which were verified by GPC as monomeric, dimeric, and trimeric fractions by elution volumes centered at 55, 46, and 44 mL, respectively. This is in good agreement with the GPC of the raw urushi exudates. Thus, the raw urushi exudate was calculated to contain approximately 84% monomeric fraction and 16% of oligomeric fractions, the latter including dimeric and trimeric fractions but not polymeric fraction.

Separation of Monomeric, Dimeric, and Trimeric Fractions from Raw Urushi Exudates. As a preliminary test, the raw urushi exudates were chromatographed by thin-layer chromatography (TLC). There were six spots with R_f values of 0.79 (spot 1), 0.65 (spot 2), 0.49 (spot 3), 0.14 (spot 4), 0.08 (spot 5), and 0.01 (spot 6). Except for spot 3, the other spots were similar in size. In contrast, spot 3 was the largest, about 10 times greater than the others. Spot 1 was extracted, and the ^{1}H NMR spectrum of the resulting extract did not show the presence of phenyl protons. In addition, its IR spectrum showed the presence of a carboxylic acid ester. Therefore, it must be one of the fragrance substances present in the raw urushi exudates (*2*, *3*). The extracts of spots 2 and 3 showed ^{1}H and ^{13}C NMR spectra characteristic of monomeric urushiols. Spots 4 and 5 could not be extracted, because they were in too-small

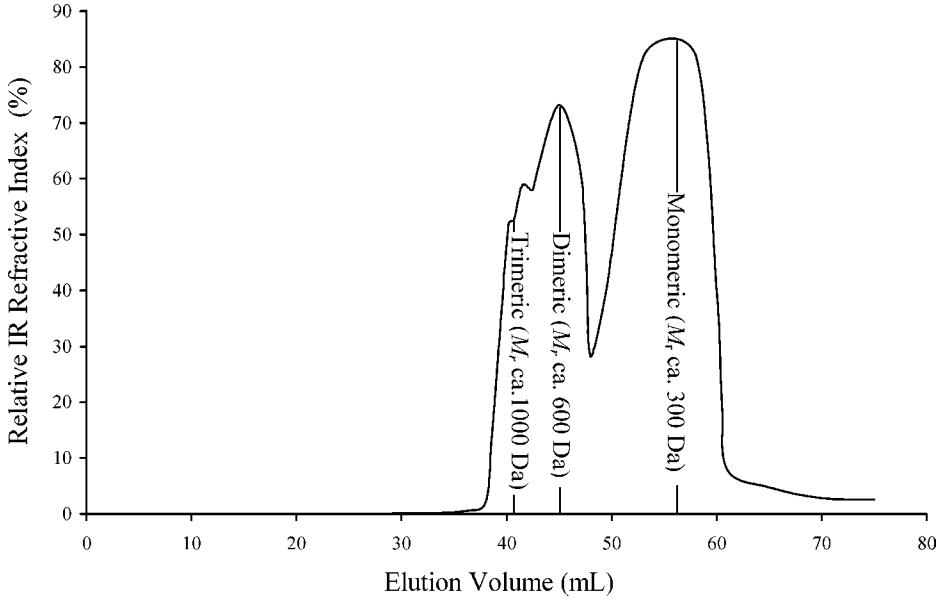

Figure 1. Molecular weight distribution of raw urushi by GPC; M_r, average relative molecular mass.

amounts. The extract of spot 6 was also rather small; we were only able to record a ^{1}H NMR spectrum that indicated it is likely polymeric urushiols. Therefore, the raw urushi exudates were fractionated by column chromatography with recycle HPLC with chloroform as eluent to separate monomeric, dimeric, and trimeric fractions.

Characterization of Monomeric Fraction. The major constituents of the resulting monomeric fraction were identified as 3-[8′*Z*,11′*E*,13′*Z*-pentadecatrienyl]catechol (**1**), 3-[8′*Z*,11′*Z*,-14′-pentadecatrienyl]catechol (**2**), and 3-(pentadecanyl)catechol (**3**) (**Figure 2**) by ^{1}H−^{1}H COSY and 2D ^{1}H−^{13}C HMQC and ^{1}H−^{13}C HMBC NMR spectra (data not shown). The structures for monomeric urushiols **1**−**3** were verified by HPLC analysis. In addition to compounds **1**−**3**, the monomer fraction also contained small amounts of 3-[8′*Z*,11′*E*-pentadecadienyl]catechol, 3-[8′*Z*,11′*Z*-pentadecadienyl]catechol, and 3-[8′*Z*-pentadecaenyl]catechol (*17*). In **Table 1**, ^{1}H and ^{13}C NMR spectroscopic data for compounds **1**−**3** are summarized. These results were verified by HPLC analysis of the fraction according to the procedure of Du and Oshima (*12*). In addition to compounds **1**−**3**, the results showed the presence of 3-[8′*Z*,11′*E*-pentadecadienyl]catechol, 3-[8′*Z*,11′*Z*-pentadecadienyl]catechol, and 3-[8′*Z*-pentadecaenyl]catechol in small amounts. Compound **1** is the most abundant component, approximately 50% of the monomeric fraction.

Characterization of Dimeric Fraction. Isolation of the constituents of dimeric fraction by column chromatography was not successful. As compared to the ^{1}H NMR spectrum of the monomeric fraction (**Figure 3A**), the ^{1}H NMR spectrum of the dimeric fraction (**Figure 3B**) showed signals at δ_H ranges 2.1−2.4, 2.9−4.4, 6.0−6.2, 6.37−7.00, and 7.5−9.1 ppm, which were not present in the ^{1}H NMR spectrum of the monmeric fraction. Therefore, ^{1}H−^{1}H COSY and 2D ^{1}H−^{13}C HMQC and HMBC NMR spectra were used to characterize the nature of these signals, in particular, signals A−M, in order to identify the constituents of dimeric fraction. This resulted in identification of 8′-(3″,4″-dihydroxy-5″-alkenyl)phenyl-3-[9′*E*,-11′*E*,13′*Z*-pentadecatrienyl]catechol (**4**), 14′-(3″,4″-dihydroxy-5″-alkenyl)-phenyl-3-[8′*Z*,10′*E*,12′*E*-pentadecatrienyl]catechol (**5**), 2-hydroxyl-3- or -6-alkenylphenyl ethyl ether (**6**), 14′-(4″,5″-dihydroxy-6″-alkenyl)phenyl-3-[8′*Z*,10′*E*,12′*E*-pentadecatrienyl]catechol (**7**), 15′-(2″-hydroxy-3″- or -6″-alkenyl)phenyloxy-3-[8′*Z*,11′*Z*,13′*E*-pentadecatrienyl]catechol (**8**), 14′-(5″,6″-dihydroxy-4″-alkenyl)-phenyl-3-[8′*Z*,10′*E*,12′*E*-pentadecatrienyl]catechol (**9**), 1,1′,2,2′-tetrahydroxy-6,6′-dialkenyl-4,3′-biphenyl (**10**), 1,1′,2,2′-tetrahydroxy-6,6′-dialkenyl-4,4′-biphenyl (**11**), 1,1′,2,2′-tetrahydroxy-6,6′-dialkenyl-5,4′-biphenyl (**12**), and 1,2,1′-trihydroxy-6,6′-dialkenyldibenzofuran (**13**) as the constituents (**Figure 2**). In the raw urushi exudates from *R. vernicifera* DC, the presence of compounds **10**−**13** was suggested by Du and Oshima (*12*) three decades ago on the basis of GC-MS analysis.

8′-(3″,4″-Dihydroxy-5″-alkenyl)phenyl-3-[9′*E*,11′*E*,13′*Z*-pentadecatrienyl]catechol (4). In the ^{1}H−^{1}H COSY spectrum of urushiol dimeric fraction (**Figure 4**), a signal at δ_H/δ_H 3.03-[labeled (A)]/1.55 corresponded to ^1J coupling of H-8′ and H-7′ of **4**, while the signal at δ_H/δ_C (A)/47.8 in the 2D ^{1}H−^{13}C HMQC spectrum (**Figure 5A**) corresponded to ^1J coupling of ^{1}H−^{13}C in the −CH- group at C-8′ in the side chain of **4**. Furthermore, in the 2D ^{1}H−^{13}C HMBC spectra (**Figure 5C**,**D**), the signal at δ_H/δ_C at (A)/134.5 corresponded to ^2J coupling of H-8′/C-1″ of a 3″,4″-dihydroxy-5″alkenylphenyl group, while signals at δ_H/δ_C (A)/111.6 and (A)/119.2 corresponded to ^3J coupling of ^{1}H−^{13}C at H-8′/C-2″ and H-8′/C-6″, respectively. In addition, signals at δ_H/δ_C 6.30/47.8 and 6.44/47.8 corresponded to ^4J coupling of H-5″/C-8′ and H-3″/H-8′, respectively. Furthermore, signals at δ_H/δ_C (A)/35.7 and (A)/139.7 corresponded to ^2J coupling of H-8′/C-7′ and H-8′/C-9′, respectively; the signal at δ_H/δ_C (A)/128.8 corresponded to ^3J coupling of H-8′/C10′; and the signal at δ_H/δ_C 5.37/47.8 corresponded to ^2J coupling of H-9′/C-8′. Thus, the 3″,4″-dihydroxy-5″-alkenylphenyl group should be bonded to C-8′ in the side chain of compound **1**.

14′-(3″,4″-Dihydroxy-5″-alkenyl)phenyl-3-[8′*Z*,10′*E*,12′*E*-pentadecatrienyl]catechol (5). In the ^{1}H−^{1}H COSY spectrum (**Figure 4**), the signal at δ_H/δ_H 3.28[labeled (B)]/1.25 corresponded to ^1J coupling of H-14′ and H-15′ of **5**, while the signal at δ_H/δ_C (B)/41.3 in the 2D ^{1}H−^{13}C HMQC spectrum (**Figure 5A**) corresponded to ^1J coupling of ^{1}H−^{13}C in the −CH- group at C-14′ in the side chain of **5**. Furthermore, in the 2D ^{1}H−^{13}C HMBC spectra (**Figure 5D**), the signal at δ_H/δ_C (B)/135.6 corresponded to ^2J coupling of H-14′/C-1″ of a 3″,4″-dihydroxyl-5″-alkenylphenyl group, while the signals at δ_H/δ_C (B)/111.0 and (B)/118.7 corresponded to ^3J coupling of ^{1}H−^{13}C at H-14′/C-2″ and H-14′/C-6″, respectively. In addition,

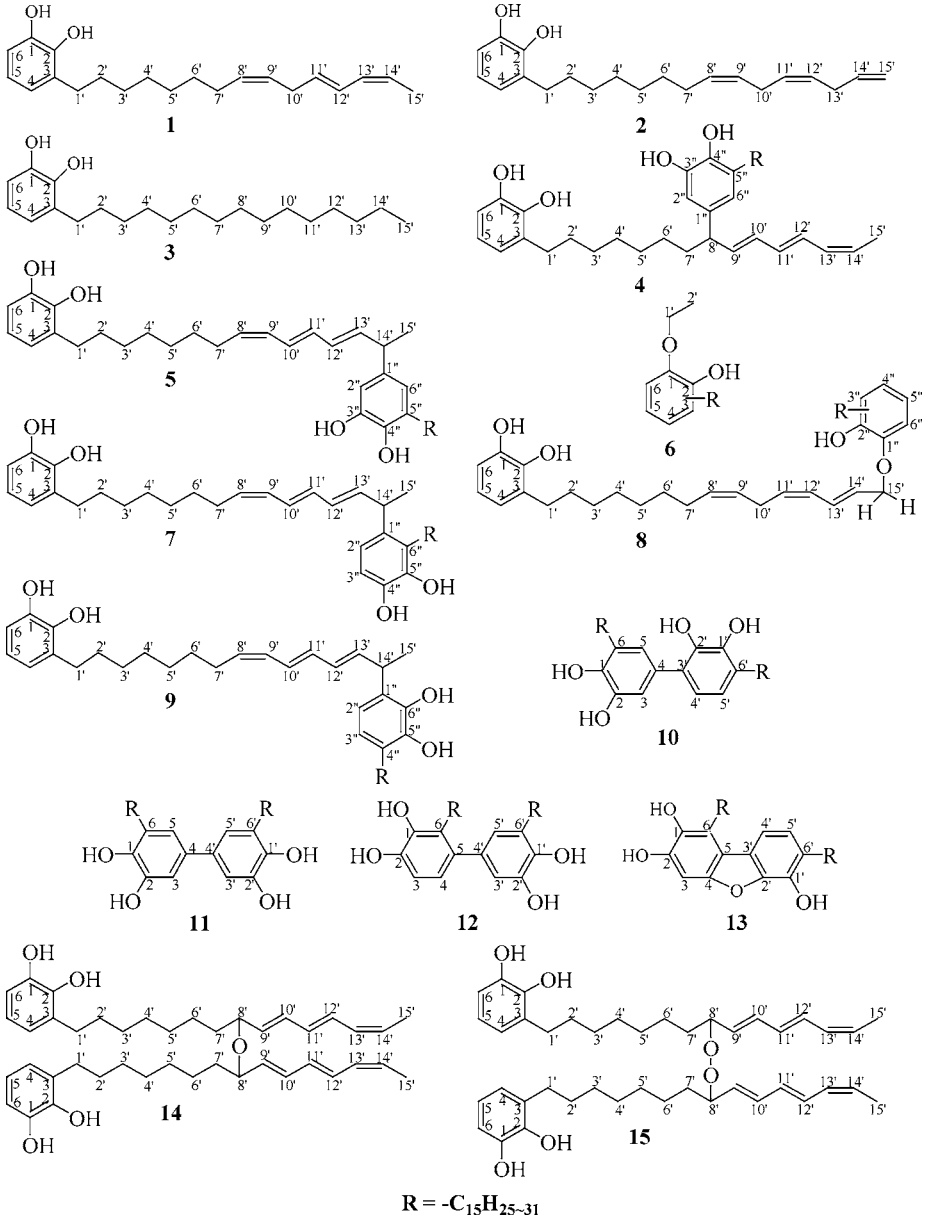

Figure 2. Structures of compounds identified in the monomic and dimeric fractions of the raw urushi.

signals at δ_H/δ_C 5.74/41.3, 6.33/41.3, and 6.46/41.3 (**Figure 5C**) corresponded to ^3J coupling of $^1H-^{13}C$ at H-13′/C-14′, H-14′/C-2″, and H-14′/C-6″, respectively. Thus, the 3″,4″-dihydroxy-5″-alkenylphenyl group should be bonded to C-14′ in the side chain of compound **2**.

2-Hydroxy-3- or -6-alkenylphenyl Ethyl Ether (6). In the $^1H-^1H$ COSY spectrum (**Figure 4**), the signal at δ_H/δ_H 3.44-[labeled (C)]/1.07 corresponded to ^1J coupling of H-14′ and H-15′ of an ethoxy group in **6**, which is confirmed by the signal at δ_H/δ_C (C)/56.1 in the 2D $^1H-^{13}C$ HMQC spectrum (**Figure 5A**), corresponding to ^1J coupling of $^1H-^{13}C$ in the −CH− group at C-14′ in the side chain of **2**. Moreover, in the 2D $^1H-^{13}C$ HMBC spectrum (**Figure 5C**), a signal at δ_H/δ_C (C)/18.0 corresponded to ^2J coupling of H-14′/C-15′, while the signal at δ_H/δ_C 1.07/56.1 corresponded to ^2J coupling of H-15′/C-14′. In addition to the absence of ^2J and ^3J couplings of both H-14′ and C-14′ with a 3,4-dihydroxy-5-alkenylphenyl group in the 2D $^1H-^{13}C$ HMBC (**Figure 5C**), the deshielding of both δ_H and δ_C of −CH-14′ indicates that the C-14′ is bonded to a phenoxy group but not with a phenyl group, to form a phenoxy ether moiety. Thus, the compound should have structure **6** that is produced by coupling of a 3,4-dihydroxyl-5-alkenylphenyl group with concomitant elimination of a 3-[8′*Z*,10′*E*,12′*Z*-tridecatrienyl]catechol fragment.

14′-(4″,5″-Dihydroxy-6″-alkenyl)phenyl-3-[8′*Z*,10′*E*,12′*E*-pentadecatrienyl]catechol (7). The $^1H-^1H$ COSY spectrum (**Figure 4**) showed a signal at δ_H/δ_H 3.57[labeled (D)]/1.25 corresponding to ^1J coupling of H-14′/H-15′, while the signal at δ_H/δ_C (D)/36.9 in the 2D $^1H-^{13}C$ HMQC spectrum (**Figure 5A**) corresponded to ^1J coupling of $^1H-^{13}C$ in the −CH− group at C-14′ in the side chain of **7**. In the 2D $^1H-^{13}C$ HMBC spectra (**Figure 5C**,**D**), signals at δ_H/δ_C 3.54(D)/3.57 and 1.25/41.3 corresponded to ^1J coupling of H-14′/C-15′ and H-15′/C-14′, respectively. Furthermore, the signal at δ_H/δ_C (D)/134.4 corresponded to ^2J coupling of H-14′/C-1″ of a 4″,5″-dihydroxy-6″-alkenylphenyl group, while signals at δ_H/δ_C (D) /127.8, (D)/116.8, and 6.44/36.9 corresponded to ^3J coupling of $^1H-^{13}C$ at H-14′/C-2″, H-14′/C-6″, and H-2″/C-14′, respectively. In addition, signals at δ_H/δ_C (D)/139.5 and 5.76/36.9 corresponded to ^1J coupling of H-14′/C-13′ and H-13′/C-14′, respectively. Thus, the 4″,5″-dihydroxy-6″-alkenylphenyl group should be bonded to C-14′ in the side chain of compound **2**.

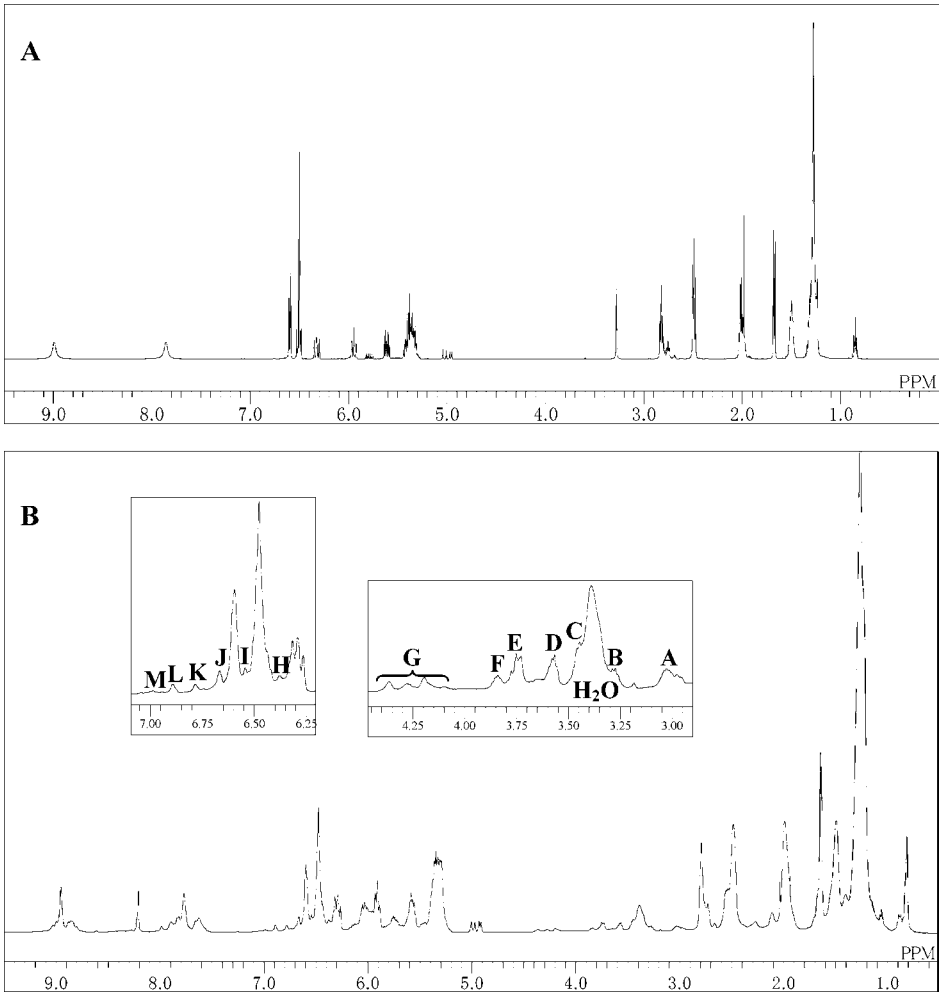

Figure 3. ^{1}H NMR spectra of (**A**) monomeric fraction and (**B**) dimeric fraction.

Table 1. ^{1}H and ^{13}C NNR Data for Major Monomeric Urushiols **1**, **2**, and **3** in DMSO-d_6

	1			**2**			**3**		
C/H no.	δ_H	multiplicity (*J*, Hz)	δ_C	δ_H	multiplicity (*J*, Hz)	δ_C	δ_H	multiplicity (*J*, Hz)	δ_C
1			143.4			142.9			142.9
2			142.4			144.7			144.7
3	6.63	ABCm	136.5	6.72		112.8	6.68		112.8
4	6.63	ABCm	120.2	6.72		118.4	6.68		118.4
5	6.63	ABCm	122.2	6.72		120.0	6.68		120.0
6			113.0			129.2			129.2
1′	2.59	t (8.2)	29.9	2.59	t (8.2)	29.5	2.55	t (7.0)	29.5
2′	1.72	m	29.2	1.72	m	29.3	1.60	m	29.3
3′	1.44	m	29.2	1.44	m	28.5	1.26	s	28.5
4′	1.44	m	29.2	1.44	m	28.6	1.26	s	28.6
5′	1.44	m	29.2	1.44	m	28.7	1.26	s	28.7
6′	1.73	m	29.2	1.73	m	28.9	1.26	s	28.9
7′	2.05	t (7.1)	27.3	2.05	t (7.11)		1.26	s	28.5
8′	5.54	td (7.1, 10.5)	130.9	5.54	td (7.1, 10.5)	126.4	1.26	s	28.6
9′	5.54	dt (10.5, 7.1)	126.8	5.54	dt (10.5, 7.1)	127.3	1.26	s	28.7
10′	2.85	dd (7.1, 6.1)	30.7	2.85	dd (7.1, 6.1)	25.0	1.26	s	28.9
11′	5.64	dd (6.1, 14.0)	132.4	5.64	dd (6.1, 14.0)	128.8	1.26	s	28.7
12′	6.42	dt (14.0, 9.7)	125.5	6.35	dt (14.0, 9.7)	12.95	1.26	s	28.9
13′	6.08	t (9.7)	129.3	2.82	*dd* (9.7, 9.7)	30.9	1.26	s	31.0
14′	6.34	dd (9.7, 7.4)	124.2	5.82	*dd* (9.7, 6.0)	129.9	1.28	m	21.9
15′	1.67	d (74)	13.1	5.06 (cis), 5.42 (trans)	dd (6.0, 9.5) (cis), dd (6.0, 16.0) (trans)	114.7	0.78	t (7.0)	13.7
OH-1	7.86	br s		7.86	s		7.76	s	
OH-2	8.98	br s		8.98	s		8.98	s	

15′-(2″-Hydroxy-3″- or -6″-alkenyl)phenyloxy-3-[8′*Z*,11′*Z*,-13′*E*-pentadecatrienyl]catechol (8). The 2D ^{1}H−^{13}C HMQC spectrum (**Figure 5A**) showed a signal at δ_H/δ_C 3.75[labeled (E)]/55.9 corresponding to ^{1}J coupling of H-15′/C-15′, while signals at δ_H/δ_C (E)/147.1 and (E)/153.0 in the 2D ^{1}H−^{13}C HMBC spectrum (**Figure 5D**) corresponded to ^{4}J coupling of H-15′/C-1″ and H-15′/C-2″ of either a 2″-hydroxy-3″- or -6″-alkenylphenoxyl group with the terminal −CH_2− group at C-15′

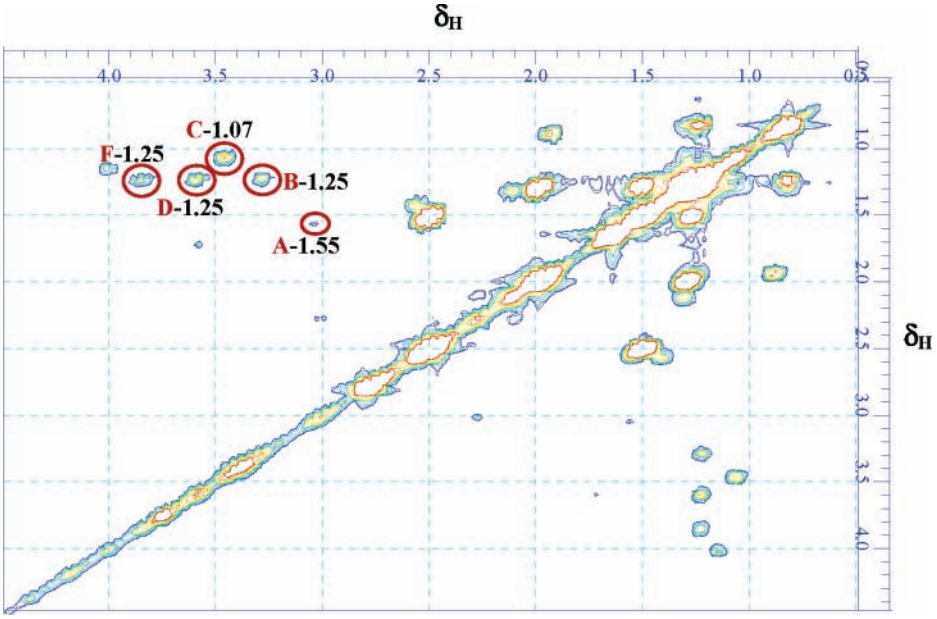

Figure 4. ^{1}H–^{1}H COSY spectrum of dimeric fraction.

in the side chain of **8**. The deshielding of both C-1″ and C-2″ indicates that the substituent at the C-15′ is a phenoxy group, not a phenyl group. This would lead to structure **8** for the compound.

14′-(5″,6″-Dihydroxy-4″-alkenyl)phenyl-3-[8′*Z*,10′*E*,12′*E*-pentadecatrienyl]catechol (9). The ^{1}H−^{1}H COSY spectrum (**Figure 4**) showed signals at δ_H/δ_H 3.85[labeled (F)]/1.25 corresponding to ^1J coupling of H-14′/H-15′, while the signal at δ_H/δ_C (F)/34.8 in the 2D ^{1}H−^{13}C HMQC spectrum (**Figure 5A**) corresponded to ^1J coupling of ^{1}H−^{13}C in the −CH− group at C-14′ in the side chain of **9**. Furthermore, in the 2D ^{1}H−^{13}C HMBC spectra (**Figure 5C**,**D**), signals at δ_H/δ_C (F)/20.1 and 1.25/34.8 corresponded to ^2J coupling of H-14′/C-15′ and H-15′/C-14′, respectively, while signals at δ_H/δ_C (F)/128.2 correspond to ^2J coupling of a H-14′/C-1″ 5″,6″-dihydroxy-4″-alkenylphenyl group, and (F)/142.3, (F)/117.4, and 6.46/34.8 correspond to ^3J coupling of ^{1}H−^{13}C at H-14′/C-2″, H-14′/C-6″, and H-2″/C-14′, respectively. In addition, signals at δ_H/δ_C (F)/138.6 and (F)/130.2 corresponded to ^1J coupling of H-14′/C-13′ and ^2J coupling of H-14′/C-12′, respectively. Thus, the 4″,5″-dihydroxy-6″-alkenylphenyl group should be bonded to C-14′ in the side chain of compound **2**.

1,1′,2,2′-Tetrahydroxy-6,6′-dialkenyl-4,3′-biphenyl (10). The 2D ^{1}H−^{13}C HMQC spectrum (**Figure 5B**) showed a signal at δ_H/δ_C 6.38[labeled (H)]/116.8 corresponding to ^1J coupling of ^{1}H−^{13}C in the −CH− groups in biphenyl ring at C-3 and C-4′ of **10**. According to Du and Oshima (*12*), the biphenyl of this type has only one broad signal for aromatic hydrogen atoms at H-3, H-5, H-4′, and H-5′ with δ_H 6.53. In the 2D ^{1}H−^{13}C HMBC spectra (**Figure 5E**), signals at δ_H/δ_C (H)/128.0 and (H)/142.0 corresponded to ^2J coupling of H-3/C-4 and H-3/C-2, respectively. This would lead to structure **10** for the compound.

1,1′,2,2′-Tetrahydroxy-6,6′-dialkenyl-4,4′-biphenyl (11). The 2D ^{1}H−^{13}C HMQC spectrum (**Figure 5B**) showed a signal at δ_H/δ_C 6.53[labeled (I)]/120.8 corresponding to ^1J coupling of ^{1}H−^{13}C in the −CH− groups of the biphenyl ring at C-3 and C-3′ of **11**, because the molecule is symmetric with respect to a plane perpendicular to the center of molecule that bisects the (C-4)−(C-4′) bond, if the nature of the alkenyl groups substituted at C-6 and C-6′ is the same or not considered. In the 2D ^{1}H−^{13}C HMBC spectra (**Figure 5E**), the signal at δ_H/δ_C (I)/147.1 corresponded to ^2J coupling of H-3/C-2 and H-3′/C-2′, while the signal at δ_H/δ_C (I)/115.2 corresponded to ^3J coupling of H-3/C-5 and H-3′/C-5′. Furthermore, the signal at δ_H/δ_C (I)/127.3 corresponded to ^2J coupling of H-3/C-4 and H-3′/C-4′, while the signal at δ_H/δ_C (I)/143.4 corresponded to ^3J coupling of H-3/C-1 and H-3′/C-1′. This would lead to structure **11** for the compound.

1,1′,2,2′-Tetrahydroxy-6′,6-dialkenyl-5,4′-biphenyl (12). The 2D ^{1}H−^{13}C HMQC spectrum (**Figure 5B**) showed signals at δ_H/δ_C 6.67[labeled (J)]/118.0, 6.78[labeled (K)]/110.8, and 6.89[labeled (L)]/114.0, corresponding to ^1J couplings of ^{1}H−^{13}C in the −CH− groups of the biphenyl ring at C-4, C-3, and C-3′ of **12**, respectively. In the 2D ^{1}H−^{13}C HMBC spectrum (**Figure 5E**), signals at δ_H/δ_C (J)/110.8 and (J)/131.8 corresponded to ^2J coupling of H-4/C-3 and H-4/C-5, respectively, while signals at δ_H/δ_C (J)/127.3 and (J)/142.0 corresponded to ^3J coupling of H-4/C-4′ and H-4/C-2, respectively. In addition, the signal at δ_H/δ_C (J)/114.3 corresponded to ^4J coupling of H-4/C-3′. Furthermore, signals at δ_H/δ_C (K)/117.9 and (K)/142.0 corresponded to ^2J coupling of H-3/C-4 and H-3/C-2, respectively, while signals at δ_H/δ_C (K)/131.8 and (K)/144.8 corresponded to ^3J coupling of H-3/C-5 and H-3/C-1, respectively. Similarly, the signals at δ_H/δ_C (L)/127.3 and (L)/142.0 corresponded to ^2J coupling of H-3′/C-4′ and H-3′/C-2′, respectively, while signals at δ_H/δ_C (L)/120.8 and (L)/144.0 corresponded to ^2J coupling of H-3′/C-5′ and H-3′/C-1′, respectively. The deshielding of the C-1, C-2, C-1′, and C-2′ indicated that these carbons are substituted by hydroxy groups. Thus, the spectroscopic data are compatible with structure **12** for the compound.

1,1′,2-Trihydroxy-6,6′-dialkenyldibenzofuran (13). In both the ^{1}H−^{1}H COSY and 2D ^{1}H−^{13}C HMQC spectra (**Figures 4** and **5B**), no signals for dibenzofuran-type structures are discernible. However, in the 2D ^{1}H−^{13}C HBQC spectra (**Figure 5E**), signals at δ_H/δ_C 6.99[labeled (M)]/143.4 and (M)/157.3 are likely to correspond to ^2J coupling of H-3/C-2 and H-3/C-4 of **13**, respectively. The deshielding of C-4 as compared to C-2 indicated that C-4 is a part of furan ring, while C-2 is substituted by a hydroxyl group. Thus, the compound should have structure **13**.

Signals in Area G of the ^{1}H NMR Spectrum of the Dimeric Fraction. In the 2D ^{1}H−^{13}C HMQC and HMBC spectra, no

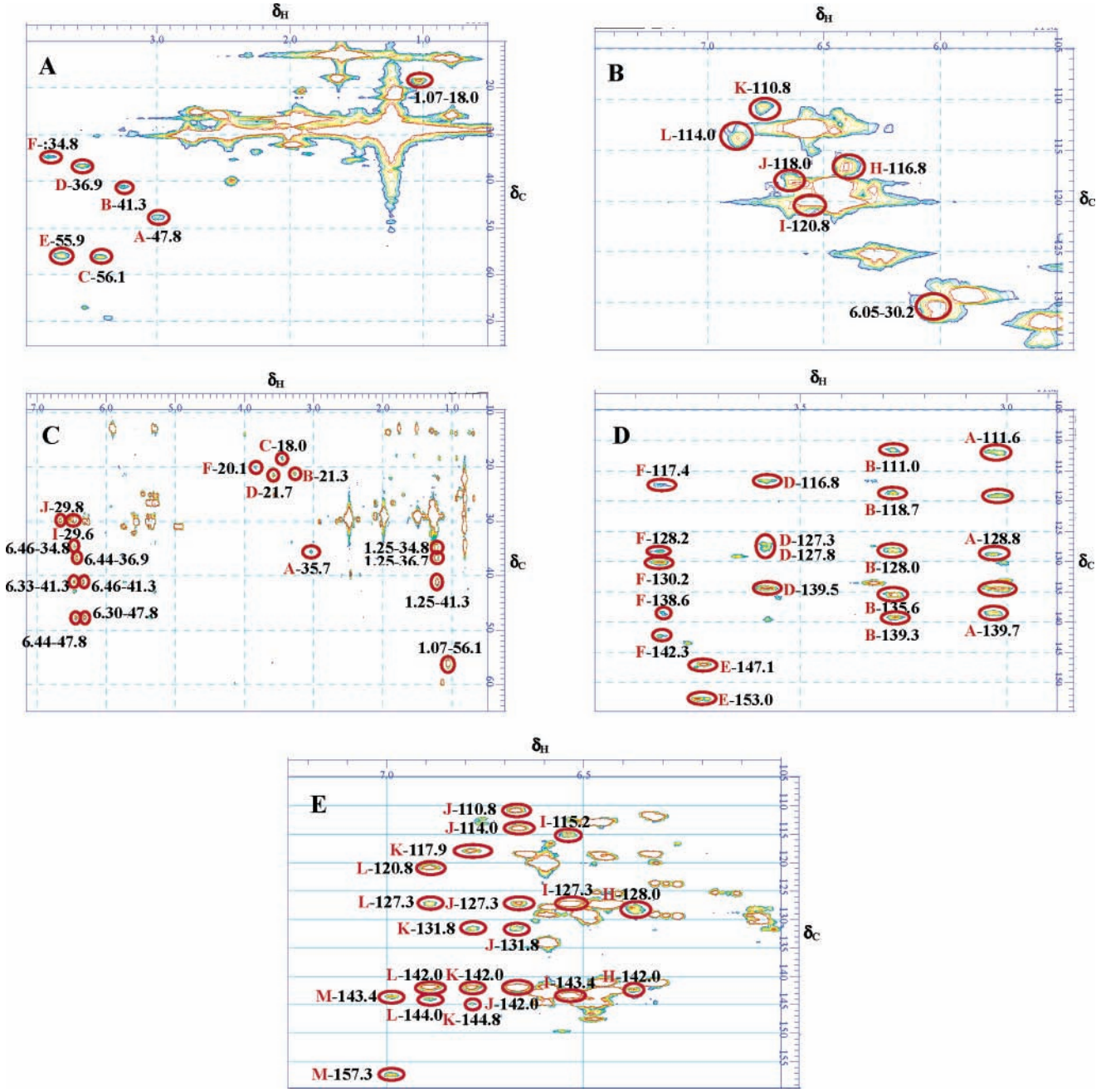

Figure 5. (**A**) HMQC spectrum of dimeric fraction (^{1}H 0.5–4.0 ppm; ^{13}C 10.0–75.0 ppm). (**B**) HMQC spectrum of dimeric fraction (^{1}H 5.5–7.5 ppm; ^{13}C 105.0–135.0 ppm). (**C**) HMBC spectrum of dimeric fraction (^{1}H 0.5–7.0 ppm; ^{13}C 10.0–65.0 ppm). (**D**) HMBC spectrum of dimeric fraction (^{1}H 2.9–4.0 ppm; ^{13}C 105.0–155.0 ppm). (**E**) HMBC spectrum of dimeric fraction (^{1}H 6.0–7.2 ppm; ^{13}C 105.0–160.0 ppm).

signals related to the signals in the area G at the δ_H range of 4.10−4.45 in the ^{1}H NMR spectrum of dimeric fraction (**Figure 3B**) are discernible. The deshielding of δ_H of protons in this region seems to indicate that hydrogen atoms in −CH− groups bonded to oxygen atoms are likely giving rise to these signals. Monomeric urushiols would undergo autoxidation in the initial stage of reactions, producing radical species involving the conjugated system produced by oxidation with the atmospheric oxygen. Oxygen would abstract a single electron from the conjugated system in the urushiols, producing corresponding radical species and superoxide ($O_2^{-\bullet}$). This would result in formation of dimeric urushiols with −HC−CH−, ether, and peroxide linkages between two monomeric urushiols, such as compounds **14** and **15.** However, further investigation is required to verify this assumption.

Reaction Mechanisms. Compounds **4** and **5** could be produced by coupling of a C-5-centered radical species of catechol radical from one of the monomeric urushiols with C-8′- and C-14′-centered radical species derived from compound **1**, with a concomitant hydrogen atom abstraction at C-10′ to form the 9′*E*,11′*E*,13′*Z* and 8′*Z*,10′*E*,12′*E* conjugate systems, respectively. Compounds **6** and **8** could be produced by radical addition of either 2-hydroxy-3-alkenylphenoxyl or 2-hydroxy-6-alkenylphenoxyl radical species to C-14′ in compounds **1** and **2**, followed by either a McLafferty-type rearrangement (*18*) resulting in elimination of a 3-[8′*Z*,10′*E*,12′*Z*-tridecatrienyl]-catechol fragment or a concomitant hydrogen atom abstraction at C-13′ to form the 8′*Z*,11′*Z*,13′*E* conjugate system, respectively. Compounds **7** and **9** could be produced by coupling of C-4- and C-6-centered catechol radical species from the mon-

omeric urushiols with a C-14′-centered radical species derived from compound **1** with a concomitant hydrogen atom abstraction at C-10′ to form the 8′*Z*,10′*E*,12′*E* conjugate system, respectively. Compounds **10** and **11** could be produced by coupling of a C-5-centered catechol radical species with a C-6-centered radical species and two C-4-centered catechol radical species from any of the monomeric urushiols, respectively. Compound **12** could be produced by coupling of a C-4-centered catechol radical species with a C-5-centered radical species from the monomeric urushiols. Compound **13** could be produced by coupling of a C-4-centered catechol radical with a C-6-centered radical species from any of the monomeric urushiols, followed by a dehydration involving the hydroxyl group at C-2′ and H-4 of the resulting intermediate catalyzed by laccase.

During transportation from the plantation to the factory for processing, the monomeric urushiols in the raw urushi exudates undergo single-electron-transferring dehydrogenative coupling to produce dimeric, trimeric, and oligomeric products, catalyzed by *Rhus* laccase under presence of atmospheric oxygen. From the urushi exudates, structures for monomeric urushiols **1**−**3** and dimeric products **4**−**14** were elucidated by using 2D-NMR spectroscopic techniques including $^{1}H-^{1}H$ COSY, and $^{1}H-^{13}C$ HMQC and HMBC sequences. Thus, the 2D-NMR spectroscopic techniques were very effective tools in the elucidation of the structures for the monomeric and dimeric constituents of raw urushi exudates.

ABBREVIATIONS USED

COSY, homonuclear shift correlation spectroscopy; HMQC, heteronuclear multiple quantum coherence; HMBC, heteronuclear multiple bond coherence; GPC, gel-permeation chromatography; DMF, *N*,*N*-dimethylformamide; DMSO-d_6, hexadeuterated dimethyl sulfoxide; PTFE, poly(tetrafluoroethylene); TMS, tetramethylsilane.

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