

## Research Article

# Preparation and Characterization of Urushiol Methylene Acetal Derivatives with Various Degrees of Unsaturation in Alkyl Side Chain

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Received 15 February 2015; Revised 16 April 2015; Accepted 16 April 2015

Academic Editor: Rong Lu

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Preparation of urushiol derivatives was carried out in response to the drug industry's increasing demand for new synthetic anticancer agents. Urushiol methylene acetal derivatives were synthesized in high yields by reaction of urushiol with methylene chloride under the catalytic action of NaOH. Four kinds of urushiol methylene acetal monomers were separated by silica-gel column and preparative HPLC, and their structures were elucidated by extensive spectroscopic methods, including 1D-NMR and 2D-NMR (<sup>1</sup>H, <sup>13</sup>C-NMR, <sup>1</sup>H-<sup>1</sup>H COSY, HSQC, and HMBC) as well as TOF-MS. They were identified as 3-[pentadecyl] benzene methylene ether (compound 1), 3-[8'-pentadecatrienyl] benzene methylene ether (compound 2), 3-[8',11'-pentadecatrienyl] benzene methylene ether (compound 3), and 3-[8',11',14'-pentadecatrienyl] benzene methylene ether (compound 4). This research provides a theoretical reference for exploration of these interesting and potentially bioactive compounds.

## 1. Introduction

Urushiols are major components of the sap of the lacquer tree (*Rhus verniciflua* Stokes, Anacardiaceae) that is widely cultivated in northeastern Asian countries including China, Korea, and Japan [1]. Urushiol is a typical phenolic compound which consists of o-dihydroxybenzene (catechol) coupled with a saturated or unsaturated alkyl side chain of 15 or 17 carbons and is an amphipathic compound [2]. Urushiol has been used as a traditional folk medicine in China and has antioxidant, antimicrobial, and anticancer activities [3]. However, urushiol can cause hypersensitive reaction, and it is sensitive to oxidation and polymerization, which reduce its activities [4]. As a result, the usefulness of urushiol as a potential therapeutic agent has been limited. Therefore, nonallergenic urushiol derivatives may be useful as bioactive compounds in the human body. It is thought that the phenolic hydroxyl group of urushiol is the main cause of

allergic reaction and polymerization [5]. Synthesis of the urushiol ester and silyl derivatives has been reported and their anticancer activities have been demonstrated [6, 7]. Recently, studies showed that acetal derivatives of phenolic compounds also have prominent antioxidant, antimicrobial, and anticancer activities, and the acetal-type groups have the advantages of including simultaneous protection of two hydroxyls and easy removal of a partial deprotection [8, 9], but, till now, there has been no report about the preparation of acetal derivatives of urushiol and its monomers.

In view of the research status of urushiol and the great potential of urushiol as a potent material in new drug research and development, our study was aimed at synthesizing urushiol methylene acetal derivatives, separating urushiol methylene acetal monomers of various unsaturated degrees, and characterizing their structures. The urushiol methylene acetal derivatives were obtained by linking methylene acetal chains to the two adjacent hydroxide groups of urushiol,

and urushiol methylene acetal monomers of 0–3 degrees of unsaturation were separated by silica-gel column and preparative high performance liquid chromatography. The structures of the urushiol methylene acetal monomers with 0–3 degrees of unsaturation were elucidated by extensive spectroscopic methods, including 1D-NMR and 2D-NMR ( $^1\text{H}$ ,  $^{13}\text{C}$ -NMR,  $^1\text{H}$ - $^1\text{H}$  COSY, HSQC, and HMBC) as well as TOF-MS and chemical analysis. Our studies provide potentially important information for further development of new urushiol derivatives and provide new leading compounds for the research on clinical drugs.

## 2. Experiments

**2.1. General Experimental Procedures.** IR spectra were measured on a Horiba FT-710 spectrophotometer. NMR spectra were recorded at 303 K on a Bruker AV-500 NMR ( $^1\text{H}$  NMR, 500 MHz;  $^{13}\text{C}$  NMR, 125 MHz) instrument with TMS in  $\text{CDCl}_3$  as the internal standard. The 2D-NMR, HSQC, HMBC, and  $^1\text{H}$ - $^1\text{H}$  COSY experiments were performed using standard Bruker pulse sequences. The TOF-MS experiment was performed on an Agilent orthogonal TOF-MS system equipped with an ESI source. The GC-MS system consisted of an Agilent 6890 N gas chromatograph and an Agilent 5973 N mass spectrometer. Column chromatography was performed on silica gel (100–200  $\mu\text{m}$ , Qingdao Marine Chemical Co., Ltd., China). Preparative HPLC was performed using a Shimadzu LC-20A instrument. Thin-layer chromatography (TLC) was performed on precoated silica gel GF254 plates (Qingdao Marine Chemical Co.).

**2.2. Materials.** Urushiol was obtained by extraction of the sap of a Chinese lacquer tree with acetone and removal of the solvent with vacuum concentration. All solvents and reagents were purchased from Nanjing Jitian Chemical Reagents Company (Nanjing, China) and were of analytical grade.

**2.3. Synthesis of Urushiol Methylene Acetal Derivatives.** This compound was prepared by a modified procedure based on the method of Werschkun and Thiem [10]. A 2 L, 4-neck round bottom flask (equipped with an  $\text{N}_2$  inlet, overhead magnetic stir drive, addition funnel, thermocoupler, and condenser) was filled with 100 mL  $\text{CH}_2\text{Cl}_2$  and 300 mL DMSO, heated to  $110^\circ\text{C}$ , and refluxed for 0.5 h; then, 100 g urushiol in 500 mL DMSO via one addition funnel and NaOH (40 g) in 100 mL water via another addition funnel were simultaneously added to the flask over 1 hour. The temperature was kept at  $110^\circ\text{C}$  for 3 h; after completion of the reaction, the mixture was diluted with water and stirred for 30 min at room temperature. It was then extracted with petroleum ether (3  $\times$  1L), and the combined petroleum ether layer was washed with water (3  $\times$  500 mL), dried with anhydrous  $\text{Na}_2\text{SO}_4$ , and concentrated in a rotary evaporator to give the crude urushiol methylene acetal derivatives.

**2.4. Separation of Urushiol Methylene Acetal Monomers.** Crude urushiol methylene acetal derivatives (60 g) were subjected to silica gel column chromatography (diameter

TABLE 1:  $^{13}\text{C}$  NMR spectral data of compounds 1, 2, 3, and 4 in pyridine- $d_5$  ( $\delta$  ppm).

Carbon	1	2	3	4
1	146.5	146.9	146.8	146.9
2	145.3	145.4	145.5	145.4
3	124.4	129.5	129.4	129.4
4	122.5	124.4	124.5	124.5
5	121.2	122.2	122.5	122.2
6	116.2	116.5	116.2	116.5
1'	29.7	29.8	29.7	29.8
2'	29.6	29.7	29.6	29.7
3'	29.6	29.6	29.6	29.6
4'	29.6	29.5	29.6	29.6
5'	29.5	29.5	29.5	29.5
6'	29.4	29.4	29.4	29.4
7'	29.3	27.4	27.3	27.3
8'	29.3	130.2	130.4	130.5
9'	29.3	130.4	128.6	127.7
10'	29.4	27.6	26.4	25.8
11'	29.3	29.3	128.5	127.5
12'	29.5	29.5	130.6	129.8
13'	31.9	31.9	27.9	26.7
14'	22.6	22.6	22.6	114.8
15'	14.1	14.1	13.9	136.9
-O-CH <sub>2</sub> -O-	76.5	77.0	77.2	77.4

60 mm  $\times$  length 800 mm) and eluted with a stepwise gradient mixture of EtOAc-Pet. ether (1:99; 2:98; 3:97; 5:95; 6:94; 8:92; 10:90). Fractions of 100 mL each were collected and controlled by TLC examination, and fractions with similar  $R_f$  values were combined, yielding three major fractions (A–C). Fraction A (15 g) was subjected to preparative HPLC (Hypersil ODS-2, 250  $\times$  10 mm i.d.,  $\text{CH}_3\text{CN}$ - $\text{H}_2\text{O}$  (95:5, v/v), 4 mL/min) to afford compounds 1, 2, 3, and 4.

**2.5. Compound 1 (3-[Pentadecyl] Benzene Methylene Ether).** Compound 1 is a colorless oil: IR (KBr)  $\gamma_{\text{max}}$  ( $\text{cm}^{-1}$ ): 1640, 1052;  $^1\text{H}$  (500 MHz, DMSO- $d_6$ ) and  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ) (see Tables 1 and 2); TOF-MS ( $m/z$ ): 331 [ $\text{M}-\text{H}$ ] $^-$ , 135 [ $\text{M}-\text{C}_{14}\text{H}_{29}$ ], 105 [ $\text{M}-\text{C}_{15}\text{H}_{31}-\text{CH}_2-2\text{H}$ ], and 77 [ $\text{M}-\text{C}_{15}\text{H}_{31}-\text{CH}_2\text{O}_2 + 2\text{H}$ ] (calculated for  $\text{C}_{22}\text{H}_{36}\text{O}_2$ , 332).

**2.6. Compound 2 (3-[8'-Pentadecatrienyl] Benzene Methylene Ether).** Compound 2 is a colorless oil: IR (KBr)  $\gamma_{\text{max}}$  ( $\text{cm}^{-1}$ ): 1640, 1598, and 1052;  $^1\text{H}$  (500 MHz, DMSO- $d_6$ ) and  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ) (see Tables 1 and 2); TOF-MS ( $m/z$ ): 329 [ $\text{M}-\text{H}$ ] $^-$ , 148 [ $\text{M}-\text{H}-\text{C}_{13}\text{H}_{25}$ ], 135 [ $\text{M}-\text{C}_{14}\text{H}_{27}$ ], 105 [ $\text{M}-\text{C}_{15}\text{H}_{29}-\text{CH}_2-2\text{H}$ ], 91 [ $\text{M}-\text{C}_{15}\text{H}_{29}-\text{CH}_2-\text{O}$ ], and 77 [ $\text{M}-\text{C}_{15}\text{H}_{29}-\text{CH}_2\text{O}_2 + 2\text{H}$ ] (calculated for  $\text{C}_{22}\text{H}_{34}\text{O}_2$ , 330).

**2.7. Compound 3 (3-[8',11'-Pentadecatrienyl] Benzene Methylene Ether).** Compound 3 is a colorless oil: IR (KBr)  $\gamma_{\text{max}}$  ( $\text{cm}^{-1}$ ): 1630, 1598, and 1052;  $^1\text{H}$  (500 MHz, DMSO- $d_6$ ) and

TABLE 2:  $^1\text{H}$  NMR spectral data of compounds 1, 2, 3, and 4 in pyridine- $d_5$  ( $\delta$  in ppm,  $J$  in  $\text{Hz}$ ).

Carbon	1	2	3	4
1				
2				
3				
4	6.72(m)	6.77(m)	6.79(m)	6.79(m)
5	6.68(m)	6.69(m)	6.70(m)	6.71(m)
6	7.25(s)	7.27(s)	7.28(s)	7.27(s)
1'	2.56(t, $J = 7.50$ )	2.61(t, $J = 7.50$ )	2.59(t, $J = 7.50$ )	2.61(t, $J = 7.50$ )
2'	1.60(m)	2.61(m)	1.63(m)	1.77(m)
3'	1.58(m)	2.58(m)	1.60(m)	1.74(m)
4'	1.58(m)	2.58(m)	1.53(m)	1.33(m)
5'	1.58(m)	2.55(m)	1.48(m)	1.60(m)
6'	1.58(m)	2.55(m)	1.46(m)	1.65(m)
7'	1.30(m)	2.55(m)	2.56(m)	2.09(m)
8'	1.30(m)	5.38(t, $J = 3.5$ )	5.44(t, $J = 4.5$ )	5.68(t, $J = 4.0$ )
9'	1.25(m)	5.35(t, $J = 4.0$ )	5.39(t, $J = 4.0$ )	5.65(t, $J = 4.0$ )
10'	1.25(m)	2.04(m)	2.81(m)	2.89(m)
11'	1.25(m)	1.65(m)	5.37(t, $J = 5.0$ )	5.48(t, $J = 3.5$ )
12'	1.25(m)	1.63(m)	5.34(t, $J = 4.5$ )	5.46(t, $J = 4.0$ )
13'	1.25(m)	1.33(m)	2.06(m)	2.84(m)
14'	1.25(m)	1.31(m)	1.41(m)	5.42(m)
15'	0.87(t, $J = 6.3$ )	0.92(t, $J = 6.3$ )	0.95(t, $J = 6.3$ )	5.39(d, $J = 5.0$ )
-O-CH <sub>2</sub> -O-	5.92(s)	5.94(s)	5.94(s)	5.99(s)

$^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ) (see Tables 1 and 2); TOF-MS ( $m/z$ ): 327  $[\text{M}-\text{H}]^-$ , 175  $[\text{M}-\text{C}_{11}\text{H}_{19}-2\text{H}]$ , 161  $[\text{M}-\text{C}_{12}\text{H}_{21}-2\text{H}]$ , 148  $[\text{M}-\text{H}-\text{C}_{13}\text{H}_{23}]$ , 135  $[\text{M}-\text{C}_{14}\text{H}_{25}]$ , 91  $[\text{M}-\text{C}_{15}\text{H}_{27}-\text{CH}_2-\text{O}]$ , and 77  $[\text{M}-\text{C}_{15}\text{H}_{27}-\text{CH}_2\text{O}_2 + 2\text{H}]$  (calculated for  $\text{C}_{22}\text{H}_{32}\text{O}_2$ , 328).

2.8. *Compound 4 (3-[8',11',14'-Pentadecatrienyl] Benzene Methylene Ether)*. Compound 4 is a colorless oil: IR (KBr)  $\gamma_{\text{max}}$  ( $\text{cm}^{-1}$ ): 1630, 1598, and 1052;  $^1\text{H}$  (500 MHz, DMSO- $d_6$ ) and  $^{13}\text{C}$  NMR (125 MHz, DMSO- $d_6$ ) (see Tables 1 and 2); TOF-MS ( $m/z$ ): 325  $[\text{M}-\text{H}]^-$ , 135  $[\text{M}-\text{C}_{14}\text{H}_{23}]$ , 105  $[\text{M}-\text{C}_{15}\text{H}_{25}-\text{O}]$ , 91  $[\text{M}-\text{C}_{15}\text{H}_{25}-\text{CH}_2-\text{O}]$ , and 79  $[\text{M}-\text{C}_{15}\text{H}_{25}-\text{CH}_2\text{O}_2 + 4\text{H}]$  (calculated for  $\text{C}_{22}\text{H}_{30}\text{O}_2$ , 326).

### 3. Result and Discussion

3.1. *Synthesis of Urushiol Methylene Acetal Derivatives*. Catechol methylene acetal is an important pharmaceutical intermediate that is vital for the synthesis of quinolones; it is traditionally synthesized through a nucleophilic substitution reaction of catechol with methylene chloride under the catalytic action of NaOH [11]. Urushiol is a typical phenolic compound possessing a catechol structure and an alkyl side chain of 15 or 17 carbons [12]; it has two adjacent phenolic hydroxyl groups on the benzene ring, so a practical method for preparation of catechol methylene acetal is desirable for natural product synthesis and process chemistry of urushiol. It is well known that the phenolic hydroxyl groups of urushiol can cause skin problems and is easy to oxidate [13], so it is necessary to modify and protect the phenolic hydroxyl

groups of urushiol. Because acetal-type protecting groups possess significant advantages, such as simultaneous protection of two hydroxyls, easy removal, and the possibility of a partial deprotection [14], the preparation of urushiol methylene acetal can not only increase the stability but also preserve the original biological activity of urushiol.

Conversion of urushiol to the urushiol methylene acetal consists of three steps, and the reaction mechanism is shown in Figure 1. First, the two adjacent phenolic hydroxyl groups of urushiol lose two hydrogen ions in the presence of NaOH, resulting in formation of catechol anions; secondly, catechol anions react with methylene chloride by bimolecular nucleophilic substitution with NaOH being the base to give the intermediate product of catechol methyl chloromethyl ether anions; finally, the intramolecular substitution reaction of the intermediate product gives the final product of urushiol methylene acetal.

To obtain the best yields of urushiol methylene acetal, the reaction conditions including reaction time and reaction temperature were optimized; the results showed that the best reaction time is 2-3 h, the best reaction temperature is 105-115°C, and the yield of urushiol methylene acetal can reach over 80%. In addition, during the reaction process, some urushiols may oxidize and polymerize, leading to the destruction of the catechol structure, thus the reaction of polymerized urushiols will not give the product of urushiol methylene acetal, but produce by-products possessing an alcohol structure. It was reported that the yields of by-products could be reduced by preventing increases in the concentration of catechol anions by adding NaOH gradually

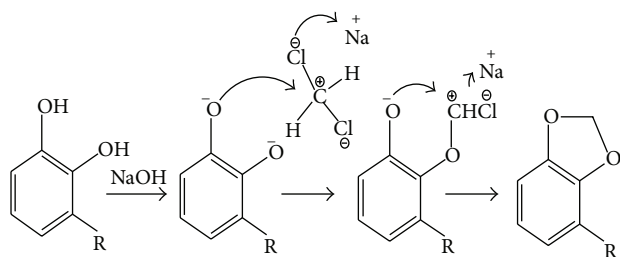


FIGURE 1: Aldolization mechanism of synthesis of urushiol methylene acetal.

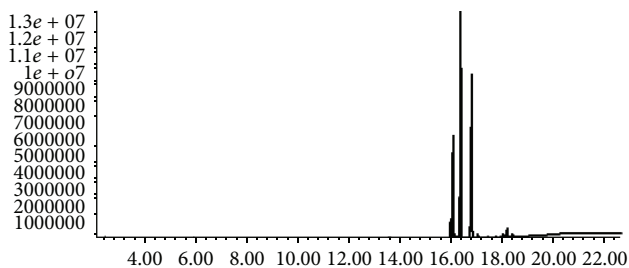


FIGURE 2: Total ion chromatography of urushiol methylene acetal by GC-MS.

and using enough DMSO solvent [15]. Furthermore, because the reaction products are composed of a water phase (NaOH solution) and an organic phase (DMSO, methylene chloride, urushiol methylene acetal, and by-products), in view of the low polarity of urushiol methylene acetal, petroleum ether was selected to extract urushiol methylene acetal from the reaction products.

After the reaction and extraction of petroleum ether, the chemical structures and yield of crude urushiol methylene acetal were determined from the IR spectrum and HPLC. The IR spectrum showed the characteristic peak of ether bonding groups at  $1100\text{ cm}^{-1}$ , and the hydroxyl groups of urushiol at  $3431\text{ cm}^{-1}$  disappeared, which indicates that the hydroxyl groups were all converted to ether-bonding groups by the reaction. The results of HPLC analysis indicate that the yield of urushiol methylene acetal was 83.5%.

**3.2. Separation of Urushiol Methylene Acetal Monomers.** The crude urushiol methylene acetal obtained was further purified by silica gel column chromatography and preparative HPLC to obtain urushiol methylene acetal monomers. First, crude urushiol methylene acetal was subjected to silica gel column chromatography and eluted by a gradient of EtOAc and Pet. Ether, yielding three major fractions (A–C) according to the TLC examination. The results of TLC analysis showed that the  $R_f$  value of fraction A is 0.5, which is similar to that of urushiol, and the  $R_f$  values of fractions B and C were 0.8–0.95, which indicated that fractions B and C had greater polarity than fraction A. It was judged that fractions B and C consisted of by-products of the reaction and polymers of urushiol. When fraction A was analyzed by GC-MS (Figure 2), the GC-MS chromatogram revealed four major peaks, whose  $m/z$  were 332, 330, 328, and 326, respectively.

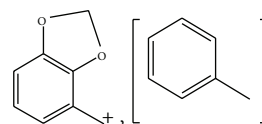


FIGURE 3: Fragment of urushiol methylene acetal under EI.

Meanwhile, the mother nucleus fragment at  $135\text{ m/z}$  was found in all four major peaks through analysis of fragment ions from samples. It was deduced that the mother nucleus fragment of  $135\text{ m/z}$  was produced by splitting of urushiol methylene acetal, and its structure is shown in Figure 3. Based on the GC-MS analysis, fraction A was demonstrated to be purified total urushiol methylene acetal. Finally, fraction A was separated by preparative HPLC affording four kinds of urushiol methylene acetal monomers: compound 1 (0.86 g), compound 2 (1.03 g), compound 3 (1.17 g), and compound 4 (0.92 g).

**3.3. Characterization of Urushiol Methylene Acetal Monomers.** The compounds' structures were elucidated mainly by 500-MHz NMR analysis, including 1D-NMR and 2D-NMR ( $^1\text{H}$ ,  $^{13}\text{C}$ -NMR,  $^1\text{H}$ - $^1\text{H}$ COSY, HSQC, HMBC, and ROESY), and TOF-MS, and by comparison with literature data [16, 17].

Compound 1 was assigned a molecular formula of  $\text{C}_{22}\text{H}_{36}\text{O}_2$ , as deduced from the  $[\text{M}-\text{H}]^-$  ion at  $m/z$  331 in negative ion mode TOF-MS. Its IR spectrum exhibited strong absorption bands at  $1640$  and  $1052\text{ cm}^{-1}$  due to a benzene ring and ether functional groups. The  $^1\text{H}$  NMR spectrum of compound 1 showed signals indicative of three protons on a benzene ring at  $\delta_{\text{H}}$  6.72, 6.68, and 7.25 (H-4, H-5, and H-6) and an alkyl group at  $\delta_{\text{H}}$  0.87–1.60 (H-1'–15'). These signals are characteristic of 1,2-dihydroxy-3-alkyl-benzene, strongly suggesting that compound 1 is an urushiol-type compound. The characteristic NMR signals for a methylene acetal group were observed in the  $^1\text{H}$  NMR spectrum of 1, with a singlet of two methylene protons at  $\delta_{\text{H}}$  5.92. The existence of the HMBC correlation between H-1' ( $\delta_{\text{H}}$  2.56), C-2 ( $\delta_{\text{C}}$  145.3), and C-4 ( $\delta_{\text{C}}$  122.5) confirmed that C-1' of the alkyl group bonded at C-3 of a benzene ring. The molecular formula and  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra indicated that the alkyl group contained 15 carbons. Based on the above-discussed results and comparison of spectral data with the literature, the structure of compound 1 was identified as 3-[pentadecyl]benzene methyl.

Compound 2 was assigned a molecular formula of  $\text{C}_{22}\text{H}_{34}\text{O}_2$ , as deduced from the  $[\text{M}-\text{H}]^-$  ion at  $m/z$  329 in negative ion mode TOF-MS. Its IR spectrum exhibited strong absorption bands at  $1640$ ,  $1598$ , and  $1052\text{ cm}^{-1}$  due to a benzene ring, olefinic, and ether functional groups. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of compound 2 were similar to those of 1 (Table 1), indicating that compound 2 was also an urushiol-type compound. The IR, molecular formula, and  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra indicated that the alkyl group of compound 2 had one double bond. The position of the double bond in the alkyl group was determined by the H-H COSY, HSQC, and HMBC spectra. In the HSQC spectrum,



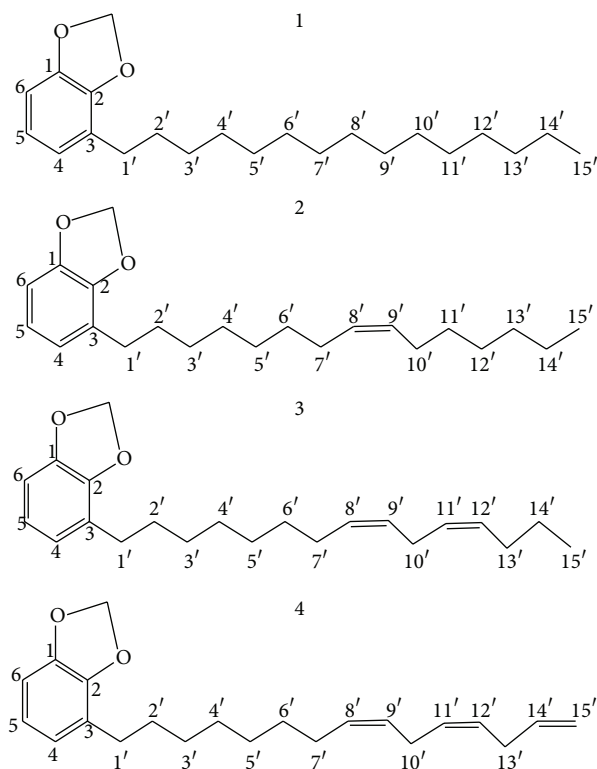


FIGURE 4: Structures of urushiol methylene acetal monomers (compounds 1, 2, 3, and 4).

the methyl proton (H-15') signal of a triplet at  $\delta_{\text{H}}$  0.92 correlated with the  $^{13}\text{C}$  signal at  $\delta_{\text{C}}$  14.1, showing that the  $^{13}\text{C}$  signal was assigned to C-15'. The cross-peak between C-15' signal at  $\delta_{\text{C}}$  14.1 and proton signal at  $\delta_{\text{H}}$  1.33 observed in the HMBC spectrum assigned the proton signal to H-13'. The H-13' signal at  $\delta_{\text{H}}$  1.33 had a cross-peak with the  $^{13}\text{C}$  signal at  $\delta_{\text{C}}$  29.3, indicating that the  $^{13}\text{C}$  signal was C-11'. The H-11' signal at  $\delta_{\text{H}}$  1.65 which correlated with the  $^{13}\text{C}$  signal at  $\delta_{\text{C}}$  29.3 from the HSQC spectrum showed a cross-peak with the  $^{13}\text{C}$  signal  $\delta_{\text{C}}$  130.4, in the HMBC spectrum, so the  $^{13}\text{C}$  signal was assigned to C-9', indicating that the double bond was located at C-9'–C-8'. In the HMBC spectrum, C-9' showed a cross-peak with the proton signal at  $\delta_{\text{H}}$  2.55 assignable to H-7' adjacent to a double bond at C-8', and C-8' showed a cross-peak with the proton signal at  $\delta_{\text{H}}$  2.04 assignable to H-10' adjacent to a double bond at C-9'.

Based on the above-discussed results, the structure of compound 2 was identified to be 3-[8'-pentadecatrienyl] benzene methylene ether (see Figure 4).

Compound 3 was assigned the molecular formula of  $\text{C}_{22}\text{H}_{32}\text{O}_2$ , as deduced from the [M–H]<sup>–</sup> ion at  $m/z$  327 in negative ion mode TOF-MS. Its IR spectrum exhibited strong absorption bands at 1630, 1598, and 1052  $\text{cm}^{-1}$  due to benzene ring, olefinic, and ether functional groups. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of compound 3 were similar to those of 1 (Table 1), indicating that compound 3 was also an urushiol-type compound. The IR, molecular formula, and  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra indicated that the alkyl group of compound 3

had two double bonds. The position of the double bond in the alkyl group was determined by the H–H COSY, HSQC, and HMBC spectra. In the HSQC spectrum, the methyl proton (H-15') signal of a triplet at  $\delta_{\text{H}}$  0.95 correlated with the  $^{13}\text{C}$  signal at  $\delta_{\text{C}}$  13.9, showing that the  $^{13}\text{C}$  signal was assignable to C-15'. The H-15' signal at  $\delta_{\text{H}}$  0.95 was coupled with the proton signal at  $\delta_{\text{H}}$  1.41 in the H–H COSY spectrum assignable to H-14'; furthermore, the H-14' signal at  $\delta_{\text{H}}$  1.41 coupled with the proton signal at  $\delta_{\text{H}}$  2.06 was assignable to protons adjacent to a double bond, and these signal relationships indicated that a double bond was located at C-12'–C-11'. The H-13' signal at  $\delta_{\text{H}}$  2.06 had a cross peak with the  $^{13}\text{C}$  signal at  $\delta_{\text{C}}$  128.5, indicating that the  $^{13}\text{C}$  signal was C-11'. The H-11' signal at  $\delta_{\text{H}}$  5.37 which correlated with the  $^{13}\text{C}$  signal at  $\delta_{\text{C}}$  128.5 from the HSQC spectrum showed a cross-peak with the  $^{13}\text{C}$  signal  $\delta_{\text{C}}$  128.6 in the HMBC spectrum, so the  $^{13}\text{C}$  signal was assigned to C-9'; this indicated that the second double bond was located at C-9'–C-8'. In the HMBC spectrum, C-9' showed a cross-peak with the proton signal at  $\delta_{\text{H}}$  2.56 assignable to H-7' adjacent to a double bond at C-8', and C-8' showed a cross-peak with the proton signal at  $\delta_{\text{H}}$  2.81 assignable to H-10' adjacent to a double bond at C-9'. Based on the above-discussed results, the structure of compound 3 was identified to be 3-[8',11'-pentadecatrienyl] benzene methylene ether (see Figure 4).

Compound 4 was assigned a molecular formula of  $\text{C}_{22}\text{H}_{30}\text{O}_2$ , as deduced from the [M–H]<sup>–</sup> ion at  $m/z$  325 in negative ion mode TOF-MS. Its IR spectrum exhibited strong absorption bands at 1630, 1598, and 1052  $\text{cm}^{-1}$  due to benzene ring, olefinic, and ether functional groups. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of compound 4 were similar to those of 1 (Table 1) indicating that compound 4 was also an urushiol-type compound. The IR, molecular formula, and  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra indicated that the alkyl group of compound 4 had three double bonds. The position of the double bonds in the alkyl group was determined by the H–H COSY, HSQC, and HMBC spectra. The proton signal of a doublet at  $\delta_{\text{H}}$  5.39 which correlated with the  $^{13}\text{C}$  signal at  $\delta_{\text{C}}$  136.9 from the HSQC spectrum showed a cross-peak with the proton signal at  $\delta_{\text{H}}$  5.42 in the H–H COSY spectrum, which indicated that one double bond was located at C-15'–C-14'. In the HMBC spectrum, C-15' also showed a cross-peak with the proton signal at  $\delta_{\text{H}}$  2.84 assignable to H-13' adjacent to a double bond at C-14'. The H-14' signal at  $\delta_{\text{H}}$  5.42 which correlated with the  $^{13}\text{C}$  signal at  $\delta_{\text{C}}$  114.8 from the HSQC spectrum had a cross-peak with the  $^{13}\text{C}$  signal at  $\delta_{\text{C}}$  129.8, indicating that the  $^{13}\text{C}$  signal was C-12' and that the second double bond was located at C-12'–C-11'. In the HMBC spectrum, C-12' also showed a cross-peak with the proton signal at  $\delta_{\text{H}}$  2.89 assignable to H-10' adjacent to a double bond at C-11'. The cross-peak between proton signal at  $\delta_{\text{H}}$  5.46 assigned to H-12' from the HSQC spectrum and proton signal at  $\delta_{\text{H}}$  5.48 observed in the H–H COSY spectrum assigned the proton signal to H-11'. The H-11' signal at  $\delta_{\text{H}}$  5.48 showed a cross-peak with the  $^{13}\text{C}$  signal  $\delta_{\text{C}}$  127.7, in the HMBC spectrum, so the  $^{13}\text{C}$  signal was assigned to C-9'. This indicated that the third double bond was located at C-9'–C-8'. In the HMBC spectrum, C-9'

also showed a cross-peak with the proton signal at  $\delta_{\text{H}}$  2.09 assignable to H-7' adjacent to a double bond at C-8'. Based on the above-discussed results, the structure of compound 4 was identified to be 3-[8',11',14'-pentadecatrienyl] benzene methylene ether (see Figure 4).

#### 4. Conclusions

We synthesized novel urushiol methylene acetal derivatives and separated four kinds of urushiol methylene acetal monomers which possess 0, 1, 2, and 3 double bonds in the alkyl side chain. This study produced valuable leading compounds to help further the design and development of more potent anticancer agents. In addition, further studies on the structure-anticancer activity relationship of urushiol methylene acetal derivatives will be carried out.

#### Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

#### Acknowledgment

This project was supported by the National Special Project of International Technology Cooperation (no. 2014DFR31300).

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