SECONDARY METABOLITES FROM THE LEAVES OF
CINNAMOMUM MACROSTEMON HAYATA

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ABSTRACT

A chemical investigation of the leaves of Cinnamomum macrostemon Hayata (Lauraceae) afforded fifteen phytochemicals including three butanolides, obtusilactone A (1), isoobtusilactone A (2), and tenuifolide B (3); three coumarins, coumarin (4), isoscopoletin (5), and scopoletin (6); two steroids, β-sitostenone (7), and β-sitosterol (8); two benzenoids, cinnamic acid (9), and eugenol (10); two lignans, (+)-yangambin (11), and (+)-syringaresinol (12); three dibenzocycloheptenes, tenuifolin (13), reticuol (14), and subamol (15). The structural elucidation was performed mainly by MS, 1D and 2D NMR spectrum data. Compounds 1-3, 5, 6, 11, 13, and 15 were found for the first time from this plant.

KEYWORDS: Cinnamomum macrostemon Hayata, Lauraceae, butanolides, dibenzocycloheptenes.

INTRODUCTION

Cinnamomum macrostemon Hayata is a medium-sized evergreen tree, and it’s endemic in Taiwan, distributed at medium altitudes throughout the island (Liao, 1996). The chemical and cytotoxic activities of several species of Cinnamomum have been evaluated in previously studies, e.g., C. camphora (Hsieh et al., 2006), C. osmophloeum (Chen et al., 2006; Hsieh et al., 2005), C. insulari-montanum (Hsieh et al., 2010), C. kotoense (Chen, 2006; Chen et al., 2008a; Chen & Hong, 2011; Cheng et al., 2010a), C. subavenium (Chen et al., 2007a; Chen & Wang, 2011a; Chen & Wang, 2011b; Chen et al., 2010a; Kuo et al., 2008b), C. reticulatum
(Chen, 2011; Chen & Yeh, 2011; Cheng et al., 2010b; Cheng et al., 2009; Chia et al., 2011), *C. tenuifolium* (Cheng et al., 2011; Lin et al., 2009), *C. burmannii* (Chen et al., 2012a; Hong et al., 2011; Li et al., 2012) and *C. philippinense* (Chen et al., 2010b; Chen et al., 2011; Cheng et al., 2012). Butanolide (Chen et al., 2007b; Chen et al., 2008b; Chen et al., 2007c; Chen et al., 2012c; Kuo et al., 2007; Kuo et al., 2008a; Liu et al., 2011; Liu et al., 2008; Wang et al., 2011b), lignanoid (Wu et al., 1994) and dibenzocycloheptenoid (Chen et al., 2010a; Cheng et al., 2010b; Chen et al., 2012a; Lin et al., 2009) compounds are three of the major classes of bioactive compounds used for their anti-tumor properties. In the course of screening for biologically and chemically novel agents from Formosan Lauraceous plants (Chen et al., 2010c; Lin et al., 2010; Lin et al., 2008; Lin et al., 2011; Shen et al., 2011; Wang et al., 2011a), *C. macrostemon* Hayata was chosen for further phytochemical investigation. Its branchlets appear to be erect, slender and glabrous; and it buds with 9 imbricate scales, scales with brown hairs in winter. Leaves appear to be chartaceous, somewhat polished above, dull beneath, ovate-lanceolate to oblong-lanceolate, 10-15 cm long, 3-4 cm wide, acuminate to obtuse apex, acute or suddenly acute at base, with 3 main veins, nerves finely prominent on both sides, not fragrant when crushed; petioles appear to be 1 cm long and are widely furrowed above (Liao, 1996). Previously, we isolated 12 compounds, including two fatty acids, one coumarin, four benzenoids, two steroids, one triterpenoid, one lignin and one dibenzocycloheptene from the twigs of this plant (Yang et al., 2011; Chen et al., 2012b). This is also being studied and published the leaves of this plant for the first time. The MeOH extract of its leaves was subjected to solvent partitioning and chromatographic separation to afford 15 pure substances. Fifteen compounds including three butanolides, obtusilactone A (1), isoobtusilactone A (2), and tenuifolide B (3); three coumarins, coumarin (4), isoscopoletin (5), and scopoletin (6); two steroids, β-sitostenone (7), and β-sitosterol (8); two benzenoids, cinnamic acid (9), and eugenol (10); two lignans, (+)-yangambin (11), and (+)-syringaresinol (12); three dibenzocycloheptenes, tenuifolin (13), reticuol (14), and subamol (15), were isolated from the leaves of *C. macrostemon*. Compounds 1-3, 5, 6, 11, 13, and 15 were found for the first time from this plant.
Figure 1: Chemical structures of compounds 1–15.

EXPERIMENTAL

General: UV spectra were obtained on a Jasco UV-240 spectrophotometer in MeCN. IR spectra were measured on a Hitachi 260-30 spectrophotometer (Hitachi, Tokyo, JP). 1H-NMR (400/500 MHz) and 13C-NMR (100 MHz), HSQC, HMBC, COSY and NOESY spectra were obtained on a Varian (Unity Plus) NMR spectrometer (Varian, CA, USA). For each sample, 128 scans were recorded with the following settings: 0.187 Hz/point; spectra width, 14400 Hz; pulse width, 4.0 μs; relaxation delay, 2s. Low-resolution ESI-MS spectra were obtained on an API 3000 (Applied Biosystems, CA, USA) and high-resolution ESI-MS spectra on a Bruker Daltonics APEX II 30e spectrometer (Bruker, Bremen, Germany). Silica gel 60 (Merck, 70~230 mesh, 230~400 mesh) was used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F-254), 0.20 mm and 0.50 mm, were used for analytical TLC and preparative TLC, respectively, and visualized with 10% H2SO4.

Plant material: The leaves of C. macrostemon Hayata were collected from Pinglin Hsiang, Taipei County, Taiwan, and November 2009. Plant material was identified by Dr. Fu-Yuan Lu (Department of Forestry and Natural Resources College of Agriculture, National Chiayi University). A voucher specimen (Cinnamo. 9) was deposited in the Department of Nutrition and Health Science, School of Medical and Health Sciences, Fooyin University, Kaohsiung City, Taiwan.

Extraction and isolation: The air-dried leaves of C. macrostemon (3.5 kg) were extracted with MeOH (10 Lx5) at room temperature and a MeOH extract (112.6 g) was obtained upon concentration under reduced pressure. The residue was placed on a silica gel column and eluted with CHCl3 gradually enriched with MeOH to afford 4 fractions. Fraction 1 (4.62 g) eluted with n-hexane–Acetone (30:1) was further purified by silica gel column chromatography using the same solvent system to obtain obtusilactone A (1) (Chen et al.,
2010a) (13 mg), isoobtusilactone A (2) (Chen et al., 2010a) (28 mg), tenuifolide B (3) (Lin et al., 2009) (4 mg), coumarin (4) (Liu et al., 2002) (8 mg), isocopoletin (5) (Lee et al., 2001) (5 mg), and scopoletin (6) (Tsukamoto et al., 1984) (10 mg). Fraction 2 (14.38 g) eluted with n-hexane–Acetone (20:1) was further separated using silica gel column chromatography and purified by preparative TLC (thin layer chromatography) to yield β-sitostenone (7) (Chen et al., 1997) (16 mg), β-sitosterol (8) (Chen et al., 1997) (75 mg), cinnamic acid (9) (Chen et al., 2009) (6 mg), and eugenol (10) (Kuo, et al., 2008b) (34 mg). Fraction 3 (7.43 g) was purified by silica gel chromatography (CH₂Cl₂–MeOH, 15:1) to give (+)-yangambin (11) (Kim, et al., 2010) (6 mg) and (+)-syringaresinol (12) (Chen et al., 1998) (8 mg). Fraction 4 (15.23 g) eluted with CH₂Cl₂–MeOH (10:1) was further purified by silica gel column chromatography using the same solvent system to obtain three dibenzocycloheptenes, tenuifolin (13) (Lin et al., 2009) (3 mg), reticuol (14) (Chia et al., 2011) (6 mg), and subamol (15) (Chen et al., 2010c) (9 mg). These compounds were obtained and characterized by comparison of their physical and spectral data with values obtained in the literature. In addition to 4, 7-10, 12 and 14, all of these compounds were found for the first time from this plant (Yang et al., 2011).

**Obtusilactone A (1):** Pale yellowish liquid; $[\alpha]^{25}_D$ -11.3 (c 0.05, CHCl₃); UV $\lambda_{max}$ (MeCN) (log ε) 225 (4.11) nm; IR (neat) $\nu_{max}$ 3400 (br, OH), 1770, 1670 (α,β-unsaturated γ-lactone), 1465, 1365, 1090 cm⁻¹; $^1$H NMR (400 MHz, CDCl₃) δ 0.88 (3H, t, $J = 7.2$ Hz, H-19), 1.25 (20H, br s, H-9~18), 1.48 (2H, m, H-8), 2.77 (2H, m, H-7), 4.67 (1H, dd, $J = 2.8$, 1.6 Hz, H-5a), 4.89, (1H, dd, $J = 2.8$, 1.6 Hz, H-5b), 5.11 (1H, br s, H-3), 6.68 (1H, td, $J = 7.6$, 2.0 Hz, H-6).

**Isoobtusilactone A (2):** Pale yellowish liquid; $[\alpha]^{25}_D$ -24.6 (c 0.05, CHCl₃); UV $\lambda_{max}$ (MeCN) (log ε) 225 (4.12) nm; IR (neat) $\nu_{max}$ 3450 (br, OH), 1770, 1670 (α,β-unsaturated γ-lactone), 1465, 1360, 1090 cm⁻¹; $^1$H NMR (400 MHz, CDCl₃) δ 0.87 (3H, t, $J = 6.8$ Hz, H-19), 1.25 (20H, br s, H-9~18), 1.52 (2H, m, H-8), 2.45 (2H, m, H-7), 4.72 (1H, dd, $J = 2.8$, 1.2 Hz, H-5a), 4.94, (1H, dd, $J = 2.8$, 1.2 Hz, H-5b), 5.12 (1H, br s, H-3), 7.07 (1H, td, $J = 7.6$, 2.4 Hz, H-6).

**Tenuifolide B (3):** Colourless oil; $[\alpha]^{25}_D$ -28.9 (c 0.05, CHCl₃); UV $\lambda_{max}$ (MeCN, log ε) 265 (4.05) nm; IR (neat) $\nu_{max}$ 3455 (br, OH), 1780, 1680 (α,β-unsaturated γ-lactone), 1290 cm⁻¹; $^1$H NMR (400 MHz, CDCl₃) δ 0.87 (3H, t, $J = 6.8$ Hz, H-20'), 1.26 (32H, br s, H-4’~19’),
1.43-1.68 (4H, m, H-2’, 3’), 3.35 (3H, s, OMe-1’), 4.12 (1H, dd, J = 7.4, 4.8 Hz, H-1’), 4.88, 5.20 (each 1H, d, J = 2.6 Hz, H-6a, b), 7.22 (1H, br s, H-4).

**Coumarin (4):** Colourless oil; UV $\lambda_{\text{max}}$ (MeCN) 260, 330 nm; IR (neat) $\nu_{\text{max}}$ 1730, 1630 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 6.44 (1H, d, $J = 9.5$ Hz), 7.31 (1H, d, $J = 7.5$ Hz), 7.35 (1H, d, $J = 7.5$ Hz), 7.50 (1H, d, $J = 7.5$ Hz), 7.53 (1H, d, $J = 7.5$ Hz), 7.72 (1H, d, $J = 9.5$ Hz).

**Isoscopoletin (5):** White needles (EtOAc), mp 184-186 ºC; UV $\lambda_{\text{max}}$ (MeCN) 260, 315 nm; IR (neat) $\nu_{\text{max}}$ 3400 (OH), 1720 (C=O), 1630 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 3.90 (3H, s, OCH$_3$-7), 6.21 (1H, d, $J = 9.6$ Hz, H-3), 6.80 (1H, s, H-8), 7.12 (1H, s, H-5), 7.82 (1H, d, $J = 9.6$ Hz, H-4).

**Scopoletin (6):** Yellowish needles (CH$_2$Cl$_2$), mp 208-210 ºC; UV $\lambda_{\text{max}}$ (MeCN) 260, 315 nm; IR (neat) $\nu_{\text{max}}$ 3400 (OH), 1720 (C=O), 1630 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 3.95 (3H, s, OCH$_3$-6), 6.27 (1H, d, $J = 9.6$ Hz, H-3), 6.85 (1H, s, H-8), 6.92 (1H, s, H-5), 7.62 (1H, d, $J = 9.6$ Hz, H-4).

**β-Sitostenone (7):** White needles (CH$_2$Cl$_2$), mp 85-86 ºC, IR $\nu_{\text{max}}$ 1675, 1620, 1450, 1375 cm$^{-1}$, $^1$H NMR (400 MHz, CDCl$_3$) : $\delta$ 0.68 (3H, s, H-18), 0.81 (3H, d, $J = 6.7$ Hz, H-26), 0.84 (3H, d, $J = 7.0$ Hz, H-29), 0.94 (3H, d, $J = 6.0$ Hz, H-21), 1.02 (3H, s, H-19), 5.72 (1H, d, $J = 1.4$ Hz, H-3).

**β-Sitosterol (8):** White needles (MeOH), mp 138-140 ºC, IR $\nu_{\text{max}}$ 3400, 2900, 1625, 1450 cm$^{-1}$, $^1$H NMR (400 MHz, CDCl$_3$) : $\delta$ 0.68 (3H, s, H-18), 0.81 (3H, d, $J = 6.8$ Hz, H-26), 0.84 (3H, d, $J = 6.8$ Hz, H-27), 0.86 (3H, t, $J = 7.0$ Hz, H-29), 0.92 (3H, d, $J = 6.4$ Hz, H-21), 1.01 (3H, s, H-19), 3.53 (1H, m, H-3), 5.35 (1H, br s, H-6).

**Cinnamic acid (9):** Yellowish needles (CH$_2$Cl$_2$), mp 208-210 ºC; UV $\lambda_{\text{max}}$ (MeCN) 260, 315 nm; IR (neat) $\nu_{\text{max}}$ 1700, 1680, 1650, 980, 770 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 6.63 (1H, d, $J = 16.0$ Hz, H-2), 7.38 (3H, m, H-6, 7, 8), 7.56 (1H, d, $J = 16.0$ Hz, H-3), 7.58 (2H, m, H-5, 9).

**Eugenol (10):** Colorless oil; UV $\lambda_{\text{max}}$ (MeCN) 230, 285 nm; IR (neat) $\nu_{\text{max}}$ 3500 (OH), 1630, 1500, 1430 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 3.38 (2H, d, $J = 6.8$ Hz, H-1’), 3.89 (3H, s,
OCH₃), 5.13 (2H, m, H-3’), 5.88 (1H, s, OH), 6.03 (1H, m, H-2’), 6.75 (1H, dd, J = 8.8, 2.0 Hz, H-5), 6.76 (1H, d, J = 2.0 Hz, H-3), 6.93 (1H, d, J = 8.8 Hz, H-6).

(+)Yangambin (11): White powder; [α]D²⁵ +32.5 (c 0.50, CHCl₃); UV λmax (MeCN) 235, 275 nm; IR (neat) νmax 1610, 1500 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 3.11 (2H, m, H-1 and H-5), 3.84 (6H, s, 2 x OCH₃), 3.89 (12H, s, 4 x OCH₃), 3.93 (2H, dd, J = 9.6, 3.6 Hz, H-4axia. and H-8axia.), 4.30 (2H, dd, J = 9.6, 6.8 Hz, H-4equ. and H-8equ.), 4.75 (2H, d, J = 4.3 Hz, H-2 and H-6), 6.59 (4H, s, H-2’, H-2”, H-6’ and H-6”).

(+)Syringaresinol (12): White powder; [α]D₂⁵ +45.3 (c 0.50, CHCl₃); UV λmax (MeCN) 235, 270 nm; IR (neat) νmax 3400 (br, OH), 1610, 1500 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 3.11 (2H, m, H-1 and H-5), 3.91 (2H, dd, J = 9.2, 3.6 Hz, H-4axia. and H-8axia.), 3.91 (12H, s, 4 x OCH₃), 4.29 (2H, dd, J = 9.2, 6.8 Hz, H-4equ. and H-8equ.), 4.74 (2H, d, J = 4.3 Hz, H-2 and H-6), 5.50 (2H, s, OH), 6.59 (4H, s, H-2’, H-2”, H-6’ and H-6”).

Tenuifolin (13): White amorphous powder; UV λmax (MeCN, log ε) 235 (3.23), 255 (2.65), 290 (2.11) nm; IR (neat) νmax 3300 (br, OH), 1610, 1500, 1070, 920 (methyleneedioxy) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 2.77 (1H, dd, J = 12.8, 6.2 Hz, H-5a), 3.08 (1H, dd, J = 12.8, 8.0 Hz, H-5b), 3.84 (3H, s, OMe-3), 4.32 (1H, d, J = 13.0 Hz, H-13a), 4.49 (1H, dt, J = 13.0, 1.4 Hz, H-13b), 6.02 (each 1H, d, J = 1.6 Hz, H-10), 6.16 (1H, t, J = 8.0, 1.4 Hz, H-6), 6.76 (1H, d, J = 2.8 Hz, H-4), 6.84 (1H, dd, J = 8.4, 2.8 Hz, H-2), 7.08 (1H, s, H-12), 7.14 (1H, s, H-8), 7.38 (1H, d, J = 8.4 Hz, H-1).

Reticuol (14): White amorphous powder; UV λmax (MeCN, log ε) 235 (3.23), 255 (2.65), 290 (2.11) nm; IR (neat) νmax 3300 (br, OH), 1610, 1500, 1070, 920 (methyleneedioxy) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 2.74 (1H, dd, J = 13.0, 6.5 Hz, H-5a), 3.04 (1H, dd, J = 12.8, 8.0 Hz, H-5b), 3.88 (3H, s, OMe-3), 4.33 (1H, d, J = 13.0 Hz, H-13a), 4.49 (1H, dt, J = 13.0, 1.4 Hz, H-13b), 6.02 (each 1H, d, J = 1.6 Hz, H-10), 6.15 (1H, t, J = 7.5 Hz, H-6), 6.69 (1H, d, J = 2.5 Hz, H-4), 6.75 (1H, dd, J = 8.5, 2.5 Hz, H-2), 7.06 (1H, s, H-12), 7.12 (1H, s, H-8), 7.32 (1H, d, J = 8.5 Hz, H-1).

Subamol (15): White amorphous powder; UV λmax (MeCN, log ε) 235 (3.23), 255 (2.65), 290 (2.10) nm; IR (neat) νmax 3400 (br, OH), 1610, 1500, 1070, 920 (methyleneedioxy) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 2.78 (1H, dd, J = 12.8, 6.2 Hz, H-5a), 3.06 (1H, dd, J = 12.8, 8.0 Hz, H-5b), 3.97 (3H, s, OMe-3), 4.36 (1H, d, J = 13.0 Hz, H-12a), 4.53 (1H, dt, J = 13.0, 1.4 Hz, H-12b),
6.16 (1H, tq, J = 8.0, 1.4 Hz, H-6), 6.70 (1H, d, J = 2.8 Hz, H-4), 6.76 (1H, dd, J = 8.4, 2.8 Hz, H-2), 7.16 (1H, s, H-11), 7.17 (1H, s, H-8), 7.36 (1H, d, J = 8.4 Hz, H-1).

RESULTS AND DISCUSSION
Compounds 1-3, 5, 6, 11, 13, and 15 were found for the first time from this plant. Obtusilactone A (1), a pale yellowish liquid, also had molecular formula C_{19}H_{32}O_{3}, as deduced from FABMS. The UV spectrum of 1 showed and intense absorption band at λ 225 nm, which is typical of β-hydroxy-γ-methylene-α,β'-unsaturated-γ-lactone ring. The IR spectrum showed absorption bands for a hydroxyl group at 3400 cm⁻¹ and an α,β-unsaturated-γ-lactone ring at 1770 and 1670 cm⁻¹. The ¹H NMR spectra of 1 presented the β-hydroxy-γ-methylene-α,β'-unsaturated-γ-lactone skeleton and the Z geometry of the trisubstituted double bond [δ 6.68 (1H, td, J = 7.6, 2.0 Hz, H-6)]. The presence of a broad singlet δ 1.25 (20H, br s, H-9-18) was attributed to protons in a long methylene chain in 1. The exocyclic olefinic protons appeared at δ 4.67, 4.89 (each 1H, dd, J = 2.8, 1.6 Hz, H-5a, b) and one hydroxymethine proton was located at δ 5.11 (1H, br s, H-3). Compound 1 showed an [α]^{25}_D -11.3° (c 0.05, CHCl₃), indicating the S configuration at C-3.

Isoobtusilactone A (2), a pale yellowish liquid, also had molecular formula C_{19}H_{32}O_{3}, as deduced from FABMS. Its spectral data (IR, UV, ¹H and ¹³C NMR) were also very similar to those of 1. The large difference between them appeared for H-1’, δ 7.07 (td, J = 7.6, 2.4 Hz) in 2 versus δ 6.68 in 1, in their ¹H NMR spectra, suggesting an E-configuration for Δ^{2(6)} in 2. The ¹H NMR spectra of 2 was similar to that of obtusilactone A, indicating that 2 has the same β-hydroxy-γ-methylene-α,β'-unsaturated-γ-lactone skeleton and the same E geometry of the trisubstituted double bond [δ 7.07 (1H, td, J = 7.6, 2.4 Hz, H-6)]. The presence of a broad singlet δ 1.25 (20H, br s, H-9-18) was attributed to protons in a long methylene chain in 2. The exocyclic olefinic protons appeared at δ 4.72, 4.94 (each 1H, dd, J = 2.8, 1.2 Hz, H-5a, b) and one hydroxymethine proton was located at δ 5.24 (1H, br s, H-3). Compound 2 showed an [α]^{25}_D -24.6° (c 0.05, CHCl₃), indicating the S configuration at C-3. Thus, the structure of isoobtusilactone A was represented as 2, and elucidated as (3S, 2E)-3-hydroxy-4-methylene-2-tetradecylidene-dihydrofuran-1-one, which was further confirmed by COSY and NOESY experiments. The ¹H and ¹³C NMR data of 2 were assigned by comparison with those of 1.
Tenuifolide B (3) was isolated as a colourless oil. The molecular formula was determined a C_{26}H_{46}O_{3} by EIMS ([M]^+ m/z 406) and HREIMS. The presence of an α, β-unsaturated γ-lactone moiety was apparent from UV absorption at 265 nm. The IR peaks at 1780 and 1680 cm\(^{-1}\) supported the presence of the α,β-unsaturated γ-lactone. The \(^1\)H NMR spectrum of 3 indicated the presence of an exo methylene group and another downfield alkene proton, which is located on the β-carbon of the unsaturated lactone from its chemical shift. In addition, it showed the signals corresponding to a methoxy functionality at δ 3.35, an oxymethine proton at δ 4.12, and long chain aliphatic protons at δ 1.26 (32H, br s), and 1.42-1.68 (4H, m). The structure of 3 is similar to that of the known butanolide, 3-(1-methoxyoctadecyl)-5-methylene-5\(H\)-furan-2-one. Thus, the structure of 3 was elucidated as 3-(1-methoxy-eicosyl)-5-methylene-5\(H\)-furan-2-one. The configuration at C-1’ remains undefined, since neither the negative rotation, nor the specific rotation can be correlated to known compounds.

Tenuifolin (13) was isolated as a white, amorphous powder with a molecular formula of C_{18}H_{16}O_{4} as determined by HREIMS (obsd [M]^+ at m/z 296.1048; calcd [M]^+ 296.1049). This formula agrees with deductions from the \(^1\)H and \(^13\)C NMR data, and corresponds to 11 degrees of unsaturation. The UV spectrum contained absorption bands typical of 5\(H\)-dibenzo[a,c] cycloheptene derivatives. IR absorption peaks at 920, 1070 and 3300 cm\(^{-1}\) indicated the presence of methylenedioxy and hydroxy functionalities, respectively. The \(^1\)H NMR resonances of 13 were well dispersed in CDCl\(_3\) and displayed an ABX pattern (H-4 at δ 6.76, H-2 at 6.84, and H-1 at 7.38), a singlet at δ 7.08 for H-12, and a singlet at δ 7.14 of H-8 in the aromatic region, in addition to the methylenedioxy protons at δ 6.02, accounting for seven protons. A three proton singlet at δ 3.84 indicated the presence of the methoxy group. The C-6 methine proton (δ 6.16, tq, J = 8.0, 1.4 Hz) coupling with the neighbor C-5 and C-13 methylene protons, which appeared coupling constant at δ 2.77 (dd, J = 12.8, 6.2 Hz, H-5a), 3.08 (dd, J = 12.8, 8.0 Hz, H-5b), 4.32 (d, J = 13.0 Hz, H-13a), and 4.49 (dt, J = 13.0, 1.4 Hz, H-13b), respectively. The \(^13\)C NMR and DEPT spectra of 13 showed 18 resonances comprising one methyl, three methylene, six methine, and eight quaternary carbons. Structure 13 was also confirmed by 2D NMR experiments. A COSY correlation was observed between H-1 and H-2, and between H-5 and H-6. A triplet of quartets at δ 6.16 was assigned to H-6 and showed coupling to the nearby C-5 and C-13 methylene protons, which appeared at δ 2.77 and 3.08, and at δ 4.49, respectively.
Subamol (15) was obtained as a white amorphous powder from CHCl₃. The molecular formula was determined as a C₁₇H₁₆O₄ by EIMS ([M]⁺, m/z 284) and HREIMS. The UV spectrum of 15 contained absorption bands typical of the 5H-dibenzo[a,c]cycloheptene derivatives (Budac and Wan, 1996). The IR spectrum of 15 showed characteristic absorption bands due to the presence of hydroxyl (3400 cm⁻¹) groups. The ¹H NMR spectrum of 15 contained an ABX pattern at δ 6.70 (1H, d, J = 2.8 Hz), 6.76 (1H, dd, J = 8.4, 2.8 Hz) and 7.36 (1H, d, J = 8.4 Hz) for H-4, H-2 and H-1, a singlet at δ 7.16 for H-11, and a singlet at δ 7.17 for H-8 in the aromatic region accounting for five protons. A singlet at δ 3.97 (3H, s) was assigned to 3-OMe. This compound was assigned as the C-6 methine proton (δ 6.16 (1H, tq, J = 8.0, 1.4 Hz)) due to the coupling with the neighbor C-5 and C-12 methylene protons which appeared coupling constant at δ 2.78 (1H, dd, J = 12.8, 6.2 Hz, H-5a), 3.06 (1H, dd, J = 12.8, 8.0 Hz, H-5b), 4.36 (1H, d, J = 13.0 Hz, H-12a) and 4.53 (1H, dt, J = 13.0, 1.4 Hz, H-12b), respectively. The ¹³C NMR and DEPT experiments of 15 showed seventeen resonance lines consisting of one methyl, two methylenes, six methines, and eight quaternary carbons. The structure of 15 was also confirmed by 2D NMR experiments.

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