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Isolation and Characterization of Rosmarinic Acid Oligomers in *Celastrus hindsii* Benth Leaves and Their Antioxidative Activity

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Antioxidative compounds were isolated from the 50% methanol extract of dried leaves of *Celastrus hindsii*. Eight phenolic compounds (**1**−**8**) were finally obtained by reversed-phase high-performance liquid chromatography, and their structures were elucidated by nuclear magnetic resonance spectrometry and mass spectrometry analyses. They were the five known compounds, rutin (**1**), kaempferol 3-rutinoside (**2**), rosmarinic acid (**3**), lithospermic acid (**4**), and lithospermic acid B (**6**), and three novel oligomers of rosmarinic acid, a dimer (**5**) and two trimers (**7** and **8**). The major components in the extract were rosmarinic acid (**3**) and lithospermic acid B (**6**). These phenolic compounds were shown to have antioxidative activities against the autoxidation of methyl linoleate in bulk phase and the radical-initiated peroxidation of soybean phosphatidylcholine in liposomes. In the liposomal peroxidation, the number of phenolic hydroxyl group in each molecule was correlated with the effectiveness of antioxidative activity.

KEYWORDS: Antioxidant; lipid peroxidation; rosmarinic acid; lithospermic acid; *Celastrus hindsii*

INTRODUCTION

Celastrus hindsii Benth (Chinese bitter-sweet) is a species of the Celastraceae family, which is used as a traditional medicine for the treatment of ulcers, tumors, and inflammation in Vietnam. In the search for antitumor agents from the Celastraceae family, a number of sesquiterpenoids and triterpenoids have been isolated from the ethanol extract of the dried stems of *C. hindsii* (*1*−*3*). Among the isolated compounds, maytenfolone A and celasdine B showed potent cytotoxicity against cancer cell lines as well as anti-HIV replication activity (*2*). In Vietnam, leaves of *C. hindsii* have been used for the manufacturing of tea products as a healthy drink. However, the water-soluble components in the tea are still unknown.

The oxidative deterioration of unsaturated lipids takes place in living organisms as well as in food. An increased intake of natural antioxidants, such as vitamin C, vitamin E, and polyphenols, may therefore have a number of health effects, such as reducing the incidence of cancer and cardiovascular diseases (*4*). Many phenolic constituents are present in plant materials as secondary metabolites, and these compounds are shown to be effective antioxidants (*5*−*7*). Therefore, some antioxidative compounds might be also present in the leaves of *C. hindsii*.

This study undertook the isolation and structural elucidation of some antioxidative compounds from the leaves of *C. hindsii*. The isolated compounds have been evaluated for their inhibitory effects against the autoxidation of methyl linoleate in bulk phase and the free radical initiated peroxidation of soybean phosphatidylcholine (PC) in liposomes.

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MATERIALS AND METHODS

Materials. Fresh leaves of *C. hindsii* were collected in March 2004 in Ha-Tay province, Vietnam. The leaves were lyophilized (yield = 31%) and stored at −20 °C until experiment. *RRR*-α-Tocopherol was purchased from Sigma Chemical Co. (St. Louis, MO) and purified by reversed-phase high-performance liquid chromatography (RP-HPLC) (*8*). Methyl linoleate (Tokyo Chemical Co., Tokyo, Japan) was purified by silica gel column chromatography to be peroxide-free (*9*). Soybean L-α-PC (95%) was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL) and purified by silica gel column chromatography. The PC (≈5 g) was subjected to chromatography on a 16 × 3.5 cm inside diameter (i.d.) silica gel 60, 70−230 mesh, column (Kanto Kagaku Co., Tokyo, Japan). The column was sequentially eluted by increasing methanol concentration in mixtures of chloroform and methanol, and PC was obtained from the elution of chloroform/methanol (4:6, v/v). Methyl linoleate hydroperoxides and PC hydroperoxides (PC-OOH) were prepared as described previously (*10*, *11*). Two free radical initiators, a water-soluble 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) and a lipid-soluble 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN), were obtained from Wako Pure Chemical Industries (Osaka, Japan). All other reagents were of analytical grade.

Apparatus. Specific rotations were determined with a SEPA-300 polarimeter (Horiba, Ltd., Kyoto, Japan). Ultraviolet (UV) spectra were measured with an Ubest-30 UV−vis spectrophotometer (Japan Spectroscopic Co., Tokyo, Japan). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded with an ECA-500 FT-NMR spectrometer (JEOL, Ltd., Tokyo, Japan) with CD_3OD as the solvent and tetramethylsilane as the internal standard. ^{1}H NMR was performed at 500.16 MHz, and the ^{1}H−^{1}H chemical shift correlated (COSY) technique was employed to assign ^{1}H shifts and couplings. ^{13}C NMR was at 125.77 MHz with proton decoupling. Heteronuclear multiple-bond correlation (HMBC) and heteronuclear multiple-quantum coherence (HMQC) techniques were used to assign correlations between ^{1}H and ^{13}C signals.

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Fast-atom bombardment mass (FABMS) spectra were measured with a JMS-700/GI mass spectrometer (JEOL, Ltd., Tokyo, Japan). *m*-Nitrobenzyl alcohol or glycerin was used as the matrix. Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) mass spectra were obtained with a Shimadzu LCMS-QP8000α instrument (Shimadzu Co., Kyoto, Japan). Sample was delivered into the ion source using acetonitrile/water (35:65, v/v) containing 0.2% formic acid at 0.2 mL/min. Elemental analysis was performed by a CHN Corder MT-6 apparatus (Yanaco Co., Kyoto, Japan).

Extraction and Isolation Procedures. Dried leaves of *C. hindsii* (70.0 g) were sequentially extracted with hot hexane (1 L) and hot ethyl acetate (1 L) for 1 h. The defatted leaves were then extracted three times with 1 L of hot water/methanol (1:1, v/v) for 1 h. The solvent was removed to afford hexane extract (7.61 g), ethyl acetate extract (0.72 g), and 50% methanol extract (20.1 g), respectively. All of these extracts had antioxidative activity with >90% inhibition against methyl linoleate autoxidation in bulk phase after a 36 h incubation (each extract was added at 0.1% based on methyl linoleate). The 50% methanol extract was subjected to chromatography on a 25 × 4.8 cm i.d. Diaion HP-20 column (Mitsubishi Chemical Co., Tokyo, Japan) with stepwise elution of methanol in water (water, 10, 20, and 40% methanol; each 1 L), and four fractions were obtained: fraction I (8.42 g) from the water eluate, fraction II (2.93 g) from the 10% methanol eluate, fraction III (1.39 g) from the 20% methanol eluate, and fraction IV (2.37 g) from the 40% methanol eluate. Fractions I and II were further subjected to chromatography on an 18 × 4.0 cm i.d. ODS-A 120-S 150 silica gel column (YMC Co., Kyoto, Japan) with stepwise elution of methanol in water and then purified by HPLC. RP-HPLC was done with a 250 × 10 mm i.d. Inertsil PREP-ODS column (GL Sciences Inc., Tokyo, Japan) developed with acetic acid 0.1% in acetonitrile/water (23:77, v/v) at a flow rate of 5.0 mL/min. The eluate was monitored by an absorbance at 280 nm. Compounds **5** (29.2 mg) and **8** (167.2 mg) were isolated from fraction I, and compounds **3** (62.2 mg), **4** (24.8 mg), **6** (179.5 mg), and **7** (24.0 mg) were isolated from fraction II. Fraction IV was subjected to chromatography on an 18 × 3.4 cm i.d. silica gel 60, 70−240 mesh, column with elution by increasing the methanol concentration in mixtures of chloroform/methanol. Two fractions eluted with chloroform/methanol (70:30 and 60:40, v/v) were further purified by HPLC. RP-HPLC was done with a 250 × 10 mm i.d. Hydrosphere C18 column (YMC Co.) developed with acetonitrile/water (23:77, v/v) at a flow rate of 5.0 mL/min. Compound **1** (26.5 mg) was isolated from the fraction of chloroform/methanol (70:30, v/v), and compound **2** (35.0 mg) was from the fraction of chloroform/methanol (60:40, v/v).

In another experiment, the dried powder of *C. hindsii* leaves (1.00 g) was extracted with 30 mL of boiling water for 5 min, or the dried powder was extracted with methanol, 50% methanol, ethanol, or 50% ethanol (each 30 mL) at 50 °C for 1 h on a water bath. After the extraction, each sample was centrifuged at 2500*g* for 10 min. The supernatant was then filtered through a 0.45 μm membrane and subjected to RP-HPLC analysis.

HPLC Analysis. HPLC was carried out using a Shimadzu LC-10AV*vp* pump equipped with a Shimadzu SPD-10AV*vp* UV−vis detector (Shimadzu Co.). The extracts were separated on a 100 × 2.0 mm i.d. Hydrosphere C18 column at 40 °C, using a 15 min linear gradient of 20% acetonitrile with 0.2% formic acid to 35% acetonitrile with 0.2% formic acid at a flow rate of 0.2 mL/min. Compounds were detected by monitoring the elution at 280 nm.

Derivatization. To 10 mL of dry acetone solution containing compound **5**, **7**, or **8** (15 mg) were added methyl iodide (2 mL), tetrahydrofuran (2 mL), and potassium carbonate (100 mg), and the reaction mixture was allowed to stir at room temperature for 48 h. Each reaction mixture was then filtered to remove the salt, and the solvent was removed to yield the corresponding methylated compound (*12*).

Structures of Compounds 1−8. *Quercetin 3-O-β-*D*-rutinoside (rutin, **1**):* yellow amorphous solid; $[\alpha]^{25}_D$ −18.6 (*c* 0.07, methanol); UV (methanol) λ_{max} (log ϵ) 257 (4.45) and 356 nm (4.30); FABMS, *m*/*z* 611.17 ([M + H]$^+$, 100%); ESIMS (negative), *m*/*z* 609.05 ([M − H]$^-$, 100%).

*Kaempferol 3-O-β-*D*-rutinoside (**2**):* yellow amorphous solid; $[\alpha]^{25}_D$ −9.5 (*c* 0.31, methanol); UV (methanol) λ_{max} (log ϵ) 266 (4.41) and 349 nm (4.30); FABMS, *m*/*z* 595.17 ([M + H]$^+$, 100%); ESIMS (negative), *m*/*z* 593.05 ([M − H]$^-$, 100%).

*[[(2E)-3-(3,4-Dihydroxyphenyl)-1-oxo-2-propenyl]oxy]-3,4-dihydroxybenzenepropanoic acid (rosmarinic acid, **3**):* white-yellow amorphous solid; $[\alpha]^{25}_D$ +90.5 (*c* 0.21, methanol); UV (methanol) λ_{max} (log ϵ) 290 (4.24) and 326 nm (4.28); ESIMS (negative), *m*/*z* 358.80 ([M − H]$^-$, 100%), 719.05 ([2M − H]$^-$, 30%); APCIMS (negative), *m*/*z* 358.95 ([M − H]$^-$, 100%); ^{1}H NMR, see **Table 1**; ^{13}C NMR, see **Table 2**.

*4-[3-[1-Carboxy-2-(3,4-dihydroxyphenyl)ethoxy]-3-oxo-1-propenyl]-2-(3,4-dihydroxyphenyl)-2,3-dihydro-7-hydroxybenzofuran-3-carboxylic acid (lithospermic acid, **4**):* pale yellow-green wax; $[\alpha]^{25}_D$ +158.4 (*c* 0.36, methanol); UV (methanol) λ_{max} (log ϵ) 253 (4.29), 289 (4.23), and 309 nm (4.22); ESIMS (negative), *m*/*z* 537.05 ([M − H]$^-$, 100%); ^{1}H NMR, see **Table 1**; ^{13}C NMR, see **Table 2**.

*4-[2-[3-(3,4-Dihydroxyphenyl)-1-oxo-2-propenoxy]-2-carboxy-1-ethyl]-2-(3,4-dihydroxyphenyl)-2,3-dihydro-7-hydroxybenzofuran-3-carboxylic acid 1-carboxy-2-(3,4-dihydroxyphenyl)ethyl ester (**5**):* pale yellow-green wax; $[\alpha]^{25}_D$ +78.3 (*c* 0.30, methanol); UV (methanol) λ_{max} (log ϵ) 287 (4.39) and 331 nm (4.33); ESIMS (negative), *m*/*z* 717.05 ([M − H]$^-$, 100%); ^{1}H NMR, see **Table 1**; ^{13}C NMR, see **Table 2**. Anal. Calcd for $C_{36}H_{30}O_{16}\cdot 4H_2O$: C, 54.69; H, 4.84. Found: C, 55.27; H, 5.00. Nonamethyl derivative of **5**: FABMS (positive), *m*/*z* 883.39 ([M + K]$^+$); APCIMS (negative), *m*/*z* 843.25 ([M − H]$^-$, 50%), 621.25 (40%), and 577.20 (100%); ^{1}H NMR (acetone-d_6) δ 2.90 (m, 1H, H-7′a), 3.00 (dd, *J* = 9.2, 14.3 Hz, 1H, H-7′b), 3.04 (dd, *J* = 9.2, 14.3 Hz, 1H, H-7*′a), 3.20 (dd, *J* = 4.0, 14.3 Hz, 1H, H-7*′b), 3.64 (s, 3H, OC*H*$_3$), 3.67 (s, 3H, OC*H*$_3$), 3.70 (s, 6H, OC*H*$_3$), 3.74 (s, 3H, OC*H*$_3$), 3.78 (s, 3H, OC*H*$_3$), 3.84 (s, 3H, OC*H*$_3$), 3.85 (s, 3H, COOC*H*$_3$), 3.88 (s, 3H, COOC*H*$_3$), 4.53 (d, *J* = 6.3 Hz, 1H, H-8*), 5.19 (dd, *J* = 4.0, 9.7 Hz, 1H, H-8′), 5.39 (dd, *J* = 4.0, 9.2 Hz, 1H, H-8*′), 5.87 (d, *J* = 6.3 Hz, 1H, H-7*), 6.31 (d, *J* = 16.0 Hz, 1H, H-8), 6.66 (dd, *J* = 2.3, 8.0 Hz, 1H, H-6*′), 6.75 (d, *J* = 8.0 Hz, 1H, H-6*), 6.79 (d, *J* = 8.0 Hz, 1H, H-5*′), 6.82 (d, *J* = 2.3 Hz, 1H, H-2*′), 6.83 (d, *J* = 8.0 Hz, 1H, H-5*), 6.86 (d, *J* = 8.6 Hz, 1H, H-6′), 6.92 (d, *J* = 2.3 Hz, 1H, H-2*), 6.96 (d, *J* = 8.0 Hz, 2H, H-5, H-5′), 7.02 (dd, *J* = 1.7, 8.6 Hz, 1H, H-6), 7.20 (d, *J* = 1.7 Hz, 1H, H-2), and 7.61 (d, *J* = 16.0 Hz, 1H, H-7).

*4-[3-[1-Carboxy-2-(3,4-dihydroxyphenyl)ethoxy]-3-oxo-1-propenyl]-2-(3,4-dihydroxyphenyl)-2,3-dihydro-7-hydroxybenzofuran-3-carboxylic acid 3-[1-carboxy-2-(3,4-dihydroxyphenyl)ethyl] ester (lithospermic acid B, **6**):* pale yellow-green wax; $[\alpha]^{25}_D$ +120.4 (*c* 0.29, methanol); UV (methanol) λ_{max} (log ϵ) 287 nm (4.39); ESIMS (negative), *m*/*z* 716.95 ([M − H]$^-$, 100%); ^{1}H NMR, see **Table 1**; ^{13}C NMR, see **Table 2**.

*4-[2-[3-(3,4-Dihydroxyphenyl)-1-oxo-2-propenoxy]-2-carboxy-1-ethyl]-2-(3,4-dihydroxyphenyl)-2,3-dihydro-7-hydroxybenzofuran-3-carboxylic acid 1-carboxy-2-[2-(3,4-dihydroxyphenyl)-2,3-dihydro-7-hydroxybenzofuran-3-carboxylic acid 1-carboxy-2-(3,4-dihydroxyphenyl)ethyl ester]ethyl ester (**7**):* pale yellow-green wax; $[\alpha]^{25}_D$ +57.7 (*c* 0.27, methanol); UV (methanol) λ_{max} (log ϵ) 286 (4.52) and 329 nm (4.35); ESIMS (negative), *m*/*z* 1075.55 ([M − H]$^-$, 100%); ^{1}H NMR, see **Table 1**; ^{13}C NMR, see **Table 2**. Anal. Calcd for $C_{54}H_{44}O_{24}\cdot 6H_2O$: C, 54.73; H, 4.76. Found: C, 53.88; H, 4.94. Tridecamethyl derivative of **7**: FABMS (positive), *m*/*z* 1297.52 ([M + K]$^+$); APCIMS (negative), *m*/*z* 1257.10 ([M − H]$^-$, 10%), 1035.25 (45%), and 991.40 (100%); ^{1}H NMR (acetone-d_6) δ 3.08 (m, 6H, H-7′, H-7*′, H-7**′), 3.66 (s, 3H, OC*H*$_3$), 3.68 (s, 3H, OC*H*$_3$), 3.69 (s, 3H, OC*H*$_3$), 3.71 (s, 3H, OC*H*$_3$), 3.76 (s, 3H, OC*H*$_3$), 3.77 (s, 3H, OC*H*$_3$), 3.78 (s, 3H, OC*H*$_3$), 3.79 (s, 3H, OC*H*$_3$), 3.83 (s, 3H, OC*H*$_3$), 3.84 (s, 3H, OC*H*$_3$), 3.855 (s, 3H, COOC*H*$_3$), 3.865 (s, 3H, COOC*H*$_3$), 3.871 (s, 3H, COOC*H*$_3$), 4.30 (d, *J* = 5.7 Hz, 1H, H-8**), 4.53 (d, *J* = 5.7 Hz, 1H, H-8*), 5.21 (dd, *J* = 4.0, 9.2 Hz, 1H, H-8′), 5.36 (m, 2H, H-8*′, H-8**′), 5.87 (d, *J* = 6.3 Hz, 1H, H-7**), 5.89 (d, *J* = 5.7 Hz, 1H, H-7*), 6.32 (d, *J* = 16.0 Hz, 1H, H-8), 6.60 (d, *J* = 8.0 Hz, 1H, H-6**′), 6.63 (d, *J* = 1.7, 8.0 Hz, 1H, H-6*′), 6.85 (m, 12H, H-5, H-5′, H-6′, H-2*, H-5*, H-6*, H-5*′, H-2**, H-5**, H-6**, H-2**′, H-5**′), 7.01 (dd, *J* = 1.7, 8.0 Hz, 1H, H-6), 7.21 (d, *J* = 1.7 Hz, 1H, H-2), and 7.60 (d, *J* = 16.0 Hz, 1H, H-7).

4-[3-[1-Carboxy-2-[2-(3,4-dihydroxyphenyl)-2,3-dihydro-7-hydroxybenzofuran-3-carboxylic acid 1-carboxy-2-(3,4-dihydroxyphenyl)ethyl

Table 1. ^{1}H NMR Data for Compounds **3**–**8**[a]

proton	3	4	5	6	7	8
2	7.05 (s)		6.93 (d, 1.6)		6.93 (d, 1.7)	
5	6.78 (d, 8.6)	6.82 (d, 8.6)	6.77 (d, 9.2)	6.83 (d, 8.6)	6.76 (d, 8.2)	6.86 (d, 8.6)
6	6.95 (d, 8.6)	7.21 (d, 8.0)	6.80 (dd, 1.7, 8.0)	7.15 (d, 8.6)	6.79 (dd, 1.7, 8.0)	6.98 (d, 8.6)
7	7.55 (d, 15.5)	7.83 (d, 16.0)	7.49 (d, 16.0)	7.52 (d, 16.0)	7.49 (d, 15.5)	7.52 (d, 16.0)
8	6.27 (d, 16.0)	6.34 (d, 16.0)	6.04 (d, 16.0)	6.20 (d, 15.5)	6.05 (d, 16.0)	6.06 (d, 16.0)
2′	6.76 (s)	6.77 (s)		6.52 (d, 2.3)		
5′	6.70 (d, 8.0)	6.69 (d, 8.0)	6.77 (d, 9.2)	6.55 (d, 8.0)	6.76 (d, 8.6)	6.74 (d, 8.0)
6′	6.62 (d, 8.0)	6.63 (d, 8.0)	6.72 (d, 8.6)	6.31 (dd, 2.3, 8.6)	6.70 (d, 8.6)	6.69 (d, 8.0)
7′a	3.01 (dd, 8.6, 14.3)	3.00 (dd, 8.0, 14.3)	2.93 (m)	2.83 (dd, 9.7, 14.3)	2.94 (m)	2.90 (m)
7′b	3.10 (dd, 4.0, 14.3)	3.07 (dd, 4.0, 14.3)	2.93 (m)	3.00 (m)	2.94 (m)	2.90 (m)
8′	5.19 (dd, 4.0, 8.0)	5.17 (dd, 4.0, 8.0)	5.09 (dd, 4.0, 9.2)	5.18 (m)	5.09 (dd, 4.0, 9.2)	5.11 (dd, 4.0, 8.6)
2*		6.80 (s)	6.75 (d, 1.7)	6.77 (d, 2.3)	6.78 (d, 2.3)	6.76 (d, 2.3)
5*		6.76 (d, 8.0)	6.63 (d, 7.4)	6.750 (d, 8.6)	6.62 (m)	6.73 (d, 8.0)
6*		6.72 (d, 8.0)	6.58 (dd, 1.7, 8.0)	6.66 (dd, 2.3, 8.0)	6.62 (m)	6.65 (dd, 2.3, 9.2)
7*		5.92 (d, 4.6)	5.74 (d, 5.2)	5.86 (d, 4.6)	5.83 (d, 5.2)	5.84 (d, 4.6)
8*		4.38 (d, 4.6)	4.42 (d, 5.2)	4.36 (d, 5.2)	4.45 (d, 5.2)	4.33 (d, 4.6)
2*′			6.63 (d, 2.3)	6.749 (d, 2.3)		6.62 (d, 2.3)
5*′			6.63 (d, 7.4)	6.71 (d, 8.0)	6.62 (d, 8.6)	6.63 (d, 8.0)
6*′			6.40 (dd, 1.7, 8.0)	6.62 (dd, 2.3, 8.6)	6.39 (d, 8.6)	6.39 (dd, 2.3, 8.6)
7*′a			2.93 (m)	3.00 (m)	2.94 (m)	2.90 (m)
7*′b			3.09 (dd, 3.4, 14.3)	3.07 (dd, 4.0, 14.3)	2.94 (m)	3.08 (dd, 3.4, 14.3)
8*′			5.23 (dd, 4.0, 9.2)	5.18 (m)	5.21 (dd, 4.0, 8.6)	5.24 (dd, 3.4, 9.2)
2**					6.78 (d, 2.3)	6.76 (d, 2.3)
5**					6.72 (d, 8.6)	6.63 (d, 8.0)
6**					6.62 (m)	6.61 (dd, 1.7, 9.7)
7**					5.70 (d, 5.7)	5.74 (d, 5.2)
8**					4.23 (d, 4.6)	4.41 (d, 5.2)
2**′					6.62 (m)	6.56 (d, 2.3)
5**′					6.61 (d, 9.2)	6.57 (d, 8.0)
6**′					6.35 (d, 9.7)	6.34 (dd, 2.3, 8.6)
7**′a					2.94 (m)	2.90 (m)
7**′b					2.94 (m)	3.00 (dd, 3.4, 14.3)
8**′					5.28 (dd, 4.6, 8.0)	5.14 (dd, 3.4, 9.2)

[a] Measured in CD_3OD (multiplicity, *J* in Hz).

*ester]ethoxy]-3-oxo-1-propenyl]-2-(3,4-dihydroxyphenyl)-2,3-dihydro-7-hydroxybenzofuran-3-carboxylic acid 1-carboxy-2-(3,4-dihydroxyphenyl)ethyl ester (**8**):* yellow wax; $[\alpha]^{25}_D$ +99.1 (*c* 0.35, methanol); UV (methanol) λ_{max} (log ϵ) 287 nm (4.40); ESIMS (negative), *m*/*z* 1075.20 ([M − H]$^-$, 100%); ^{1}H NMR, see **Table 1**; ^{13}C NMR, see **Table 2**. Anal. Calcd for $C_{54}H_{44}O_{24}\cdot 6H_2O$: C, 54.73; H, 4.76. Found: C, 55.29; H, 4.66. Tridecamethyl derivative of **8**: FABMS (positive), *m*/*z* 1297.52 ([M + K]$^+$); APCIMS (negative), *m*/*z* 1257.85 ([M − H]$^-$, 10%), 1035.35 (20%), 991.30 (35%), and 577.35 (100%); ^{1}H NMR (acetone-d_6) δ 2.96 (m, 4H, H-7′, H-7*′a, H-7**′a), 3.09 (dd, *J* = 4.6, 14.9 Hz, 1H, H-7**′b), 3.17 (dd, *J* = 4.6, 14.3 Hz, 1H, H-7*′b), 3.56 (s, 3H, OCH_3), 3.62 (s, 3H, OCH_3), 3.63 (s, 3H, OCH_3), 3.68 (s, 6H, 2OCH_3), 3.70 (s, 3H, OCH_3), 3.74 (s, 6H, 2OCH_3), 3.79 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.94 (s, 9H, 3COOCH_3), 4.46 (d, *J* = 5.7 Hz, 1H, H-8*), 4.58 (d, *J* = 5.7 Hz, 1H, H-8**), 5.15 (d, *J* = 4.0, 9.2 Hz, 1H, H-8′), 5.22 (dd, *J* = 4.0, 9.2 Hz, 1H, H-8**′), 5.36 (dd, *J* = 4.0, 9.2 Hz, 1H, H-8*′), 5.85 (d, *J* = 5.7 Hz, 1H, H-7**), 5.94 (d, *J* = 5.7 Hz, 1H, H-7*), 6.14 (d, *J* = 15.5 Hz, 1H, H-8), 6.59 (dd, *J* = 1.7, 8.0 Hz, 1H, H-6**′), 6.64 (dd, *J* = 1.7, 8.0 Hz, 1H, H-6*′), 6.70 (d, *J* = 1.7 Hz, 1H, H-2**′), 6.74 (d, *J* = 8.0 Hz, 1H, H-5**′), 6.77 (d, *J* = 8.0 Hz, 1H, H-5*′), 6.87 (m, 8H, H-5′, H-6′, H-5*, H-6*, H-2*′, H-2**, H-5**, H-6**), 6.99 (d, *J* = 1.7 Hz, 1H, H-2*), 7.02 (d, *J* = 8.6, 1H, H-5), 7.07 (d, *J* = 8.6 Hz, 1H, H-6), and 7.60 (d, *J* = 16.0 Hz, 1H, H-7).

Antioxidative Activity. The antioxidative activity of each isolated compound was measured by its inhibition against methyl linoleate autoxidation in bulk phase (*13*). Methyl linoleate (294 mg, 1.0 mmol) containing the isolated compound (each 0.05 μmol; 0.005 mol %, based on methyl linoleate), α-tocopherol, or quercetin (0.005 mol %, based on methyl linoleate) was placed in a test tube (1.5 cm in diameter) and incubated at 60 °C in the dark. After intervals of 12 h of incubation, each sample (25 μL) was withdrawn and dissolved in 1.0 mL of ethanol. The peroxide value in each sample solution was determined by using the iodometric method (*9*). Methyl linoleate monohydroperoxide was used as the standard peroxide.

The antioxidative activity was also determined using PC liposomal systems. Large unilamellar liposomes containing antioxidants were prepared according to the extrusion method (*14*). Soybean PC containing the isolated compound, α-tocopherol, or quercetin (0.05 mol % or 0.025% based on PC, in case of AAPH or AMVN, respectively) was suspended by vigorous mixing for 2 min in a 50 mM sodium phosphate buffer (pH 7.4, containing 50 mM NaCl). The milky suspension was transferred into a LiposoFast apparatus (Avestin, Ottawa, Canada), extruded 21 times back and forth through a polycarbonate membrane (100 nm pore size), and diluted with the same buffer to give a final PC concentration of 10 mM. In the experiment with a water-soluble radical initiator, the liposomal suspension was added to the AAPH solution (final concentration of AAPH = 3 mM) to start lipid peroxidation. When a lipid-soluble radical initiator was used, the ethanol solution of AMVN (final concentration of AMVN = 2 mM) was mixed with PC before liposomes were prepared. In this case, the liposomes were quickly prepared at 4 °C to prevent the start of AMVN decomposition. The peroxidation was carried out at 37 °C under air with mechanical shaking. At regular intervals, an aliquot of reaction mixture (50 μL) was withdrawn and dissolved in 0.45 mL of ethanol. The amount of PC-OOH was analyzed by RP-HPLC (*14*).

RESULTS AND DISCUSSION

Structures of Isolated Compounds. The 50% methanol extract fraction of dried leaves of *C. hindsii* was analyzed by RP-HPLC (**Figure 1**). Many peaks including **1**−**8** appeared on the chromatogram detected at 280 nm. For the isolation and characterization of the detected peaks, the 50% methanol extract was subjected to Diaion HP-20 column chromatography followed by ODS-silica gel or silica gel column chromatography. Finally, the fractions containing antioxidative compounds were isolated by preparative RP-HPLC to obtain compounds **1**−**8**,

Table 2. ^{13}C NMR Data for Compounds **3**–**8**[a]

carbon	3	4	5	6	7	8
1	127.7	124.7	127.6	124.7	127.6	124.5
2	115.2	127.6	115.5	126.4	115.5	126.4
3	146.8	148.9	146.5	149.1	146.6	148.9
4	149.7	145.2	149.7	145.0	149.7	145.0
5	116.3	118.3	116.5	118.4	116.5	118.4
6	123.2	122.0	123.3	123.3	123.3	122.0
7	147.7	144.1	148.0	143.5	148.0	143.7
8	114.5	116.5	114.0	116.6	114.1	116.1
9	168.5	168.2	168.6	168.1	168.6	168.1
1′	129.4	129.3	126.9	129.0	126.9	126.6
2′	117.6	117.7	126.2	117.3	126.2	126.1
3′	146.2	146.1	148.9	145.9	149.0	148.8
4′	145.3	145.3	141.4	145.0	141.5	141.4
5′	116.5	116.4	118.1	116.4	116.7	116.4
6′	121.9	121.8	123.7	121.8	123.8	124.0
7′	38.0	38.0	34.7	37.9	34.8	34.6
8′	74.8	74.8	75.0	75.7	75.0	74.5
9′	173.6	173.5	172.7	172.7	172.7	172.6
1*		133.9	134.1	133.7	134.1	133.6
2*		113.5	113.5	113.4	113.6	113.5
3*		146.6	146.4	146.6	146.4	146.6
4*		146.7	146.6	146.7	146.6	146.7
5*		116.4	116.6	116.5	116.5	116.4
6*		118.4	118.3	118.4	118.4	118.1
7*		88.8	88.3	88.3	88.4	88.2
8*		57.6	58.2	58.0	58.3	57.7
9*		175.2	173.1	172.3	173.0	172.2
1*′			129.1	129.3	125.7	129.0
2*′			117.3	117.6	125.8	117.3
3*′			146.1	146.1	148.7	145.9
4*′			145.2	145.2	141.3	145.1
5*′			116.5	116.4	118.1	116.5
6*′			121.9	122.1	124.2	121.9
7*′			37.6	37.5	34.0	37.5
8*′			75.6	74.8	74.0	75.4
9*′			173.5	173.8	172.9	172.6
1**					133.8	134.0
2**					113.8	113.4
3**					146.4	146.4
4**					146.6	146.5
5**					116.5	116.5
6**					118.6	118.6
7**					88.4	88.3
8**					58.0	58.1
9**					173.1	173.1
1**′					129.0	128.9
2**′					117.5	117.3
3**′					146.0	146.0
4**′					145.2	145.0
5**′					118.1	116.4
6**′					122.1	121.9
7**′					37.6	37.4
8**′					75.6	75.5
9**′					173.6	173.5

[a] Measured in CD_3OD.

which correspond to peaks 1−8 in **Figure 1**, respectively. The structures of compounds **1**−**8** were characterized as follows (**Figure 2**).

Compounds **1** and **2** were obtained as yellow amorphous solids. Their structures were identified to be quercetin 3-*O*-*β*-D-rutinoside (rutin, **1**) and kaempferol 3-*O*-*β*-D-rutinoside (**2**) by comparison of their ^1H and ^{13}C NMR data with those in the literature (*15*−*17*). Rutin and kaempferol 3-*O*-*β*-D-rutinosides are major flavonoid glycosides found in a variety of plants (*15*, *18*).

Compounds **3**, **4**, and **6** were obtained as a white-yellow amorphous solid (**3**) and pale yellow-green wax (**4** and **6**). Their structures were identified to be rosmarinic acid (**3**), lithospermic acid (**4**), and lithospermic acid B (**6**), respectively, by comparison of their ^1H and ^{13}C NMR data with those reported in the literature (*19*−*26*). Rosmarinic acid (**3**), and its derivatives, **4** and **6**, are polyphenols synthesized through the phenylpropanoid pathway in plants (*21*, *27*, *28*): **3** is an ester of caffeic acid with 3,4-dihydrophenyl lactic acid, and **4** and **6** are conjugates of rosmarinic acid with caffeic acid and rosmarinic acid, respectively. Rosmarinic acid (**3**) and its derivatives, **4** and **6**, have been found in some plants, *Lithospermum ruderale* (*19*, *20*), *Perilla flutescens* (*23*), *Salvia officinalis* (*24*), *Borago officinalis* L. (*25*), and *Salvia miltiorrhizae* (*29*). The cell suspension cultures of *Lithospermun erythrorhizon* have produced a large amount of these compounds (*21*, *28*). These compounds are expected to have some biological and pharmaceutical activities (*23*, *29*−*35*): rosmarinic acid (**3**) showed antioxidative activity in the biological systems through the scavenging of superoxide anion (*23*) and antiallergic activity through the inhibition of hyaluronidase and *β*-hexoamindase (*31*); lithospermic acid (**4**) and lithospermic acid B (**6**) were potent and nontoxic inhibitors of human immunodeficiency virus type HIV-1 replication (*33*). In addition, lithospermic acid B (**6**) showed endothelium-dependent vasodilator effects (*29*) and an ameliorative effect on ischemia reperfusion induced acute renal failure in rats (*35*).

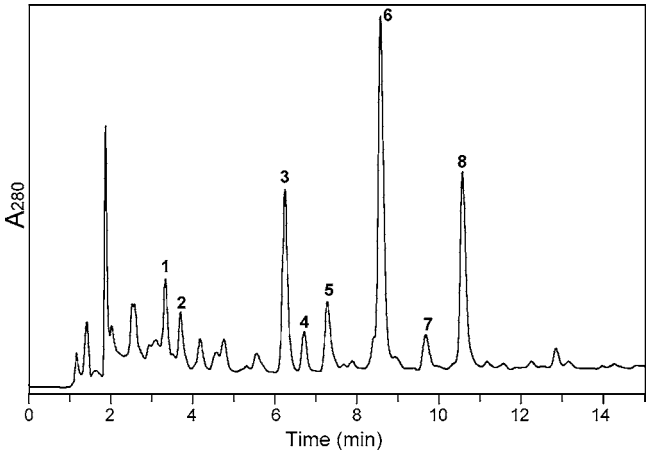

Figure 1. HPLC chromatogram of the 50% methanol extract from dried leaves of *C. hindsii*.

Compound **5** was obtained as a pale yellow-green wax. The molecular formula of **5** was the same as that of **6**, determined by low-resolution MS and elemental analyses to be $C_{36}H_{30}O_{16}$. The ^1H NMR spectrum of **5** showed signals similar to those of **6**, indicating the presence of two trans olefinic proton signals at δ 6.04 (d, $J = 16.0$ Hz, H-8) and 7.49 (d, $J = 15.5$ Hz, H-7), two sets of oxygen-bearing methine proton signals at δ 5.09 (H-8′) and 5.23 (H-8*′), methylene proton signals at δ 2.93 (H-7′a, H-7′b, and H-7*′a) and 3.09 (H-7*′b), and a pair of mutually coupled aliphatic proton signals at δ 4.42 (H-8*) and 5.74 (H-7*) (**Table 1**). The ^{13}C NMR spectrum of **5** showed 36 carbon signals, including characteristic signals of 4 carbonyls at δ 168.6 (C-9), 172.7 (C-9′), 173.1 (C-9*), and 173.5 (C-9*′), 2 olefinic carbons at δ 148.0 (C-7) and 114.0 (C-8), and 2 aliphatic carbons at δ 88.3 (C-7*) and 58.2 (C-8*) (**Table 2**). Methylation of **5** with methyl iodide afforded a nonamethyl derivative, indicating the presence of five hydroxyl and two carboxyl groups. The structural assignment was supported by HMBC analysis: the correlations were observed between the trans olefinic proton signal at δ 7.49 (H-7) and carbon signals at δ 115.5 (C-2), 123.3 (C-6), and 168.6 (C-9); between the oxygen-bearing methine proton signal at δ 5.09 (H-8′) and carbon signals at δ 126.9 (C-1′), 168.6 (C-9), 172.7 (C-9′); between the proton signal at δ 5.23 (H-8*′) and carbon signals

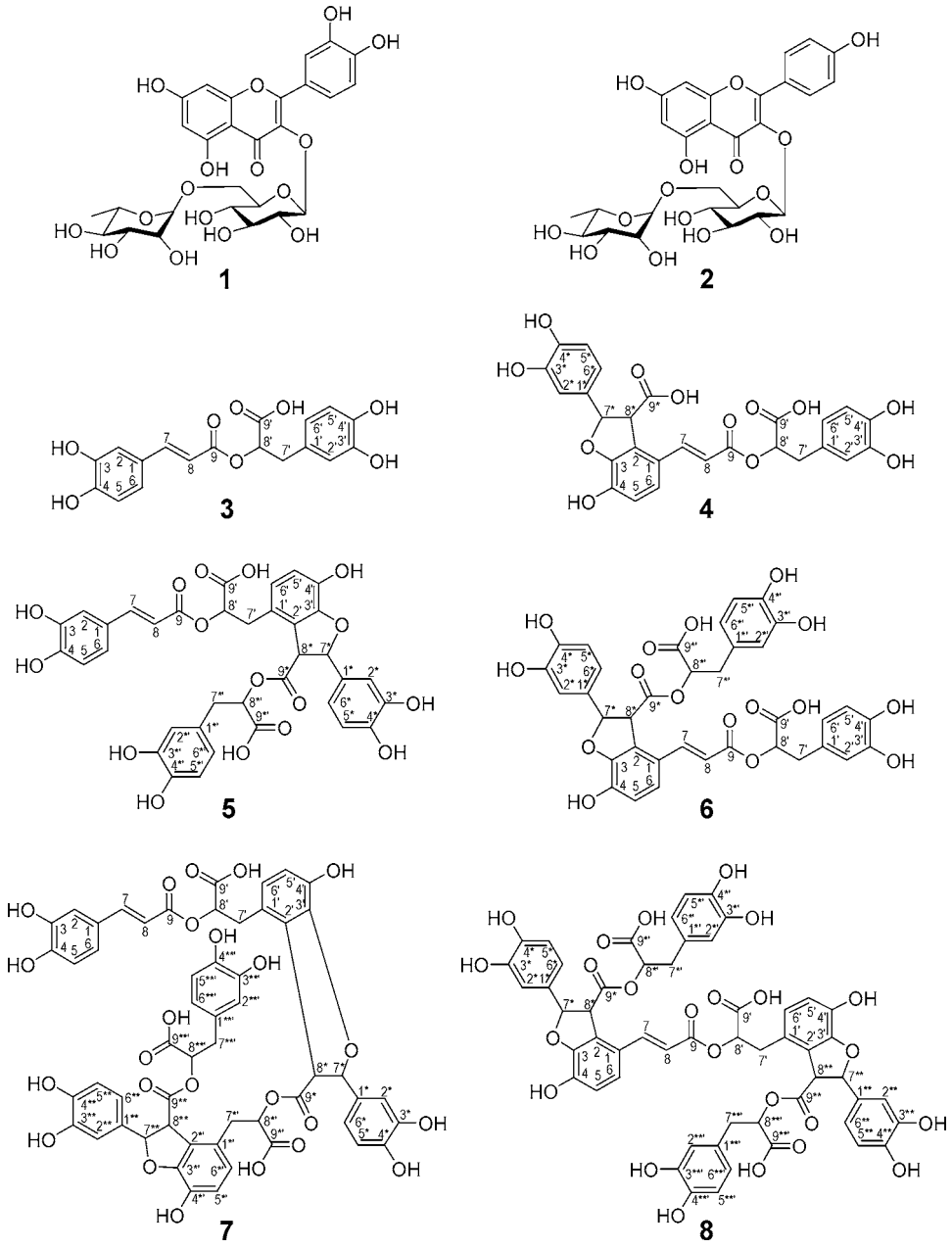

Figure 2. Structures of compounds **1**–**8** isolated from leaves of *C. hindsii.*

at δ 129.1 (C-1*′), 173.1 (C-9*), and 173.5 (C-9*′); and between the methine proton signal at δ 4.42 (H-8*) and carbon signals at δ 126.9 (C-1′), 148.9 (C-3′), and 134.1 (C-1*); and a proton signal at δ 5.74 (H-7*) was long-range coupled to carbon signals at δ 113.5 (C-2*), 118.3 (C-6*), 126.2 (C-2′), 148.9 (C-3′), and 173.1 (C-9*). Furthermore, the ^{1}H and ^{13}C NMR data were compared with those of known compounds, **3**, **4**, and **6**. The results indicate that compound **5** was a condensation product of two rosmarinic acid molecules formed via an oxidative cyclization leading to the formation of 2,3-dihydrobenzofuran ring structure, in which the C-7* and C-8* positions of the caffeic acid moiety of one rosmarinic acid are substituted by the addition of C-2 and C-3 positions of the 3,4-dihydrophenyl lactic acid moiety of the other molecule (*28*). Thus, the structure of compound **5** was determined to be 4-[2-[3-(3,4-dihydroxyphenyl)-1-oxo-2-propenoxy]-2-carboxy-1-ethyl]-2-(3,4-dihydroxyphenyl)-2,3-dihydro-7-hydroxybenzofuran-3-carboxylic acid 1-carboxy-2-(3,4-dihydroxyphenyl)ethyl ester. The stereochemistry of the C-7* and C-8* positions of the dihydrobenzofuran moiety could not be resolved.

Compounds **7** and **8** were obtained as a pale yellow-green wax (**7**) and a yellow wax (**8**). The same molecular formula of $C_{54}H_{44}O_{24}$ was assumed from the data of low-resolution MS and elemental analyses of **7** and **8**. Methylation of **7** and **8** afforded tridecamethyl derivatives, indicating the presence of 10 hydroxyl groups and 3 carboxyl groups in each molecule. The ^{1}H NMR and ^{13}C NMR spectra of **7** and **8** were similar to one another (**Tables 1** and **2**). However, differences in chemical shift were observed. The ^{1}H NMR spectrum of each compound indicates the presence of two trans olefinic proton signals [**7**, δ 6.05 (d, J = 16.0 Hz, H-8) and 7.49 (d, J = 15.5 Hz, H-7); **8**, δ 6.06 (d, J = 16.0 Hz, H-8) and 7.52 (d, J = 16.0 Hz, H-7)], three oxygen-bearing methine proton signals [**7**, δ 5.09 (H-8′), 5.21 (H-8*′), and 5.28 (H-8**′); **8**, δ 5.11 (H-8′), 5.14 (H-8**′), and 5.24 (H-8*′)], and two pairs of mutually coupled aliphatic proton signals [**7**, δ 4.23 (H-8**) and 5.70 (H-7**), and δ 4.45

Table 3. Contents of Compounds **1**–**8** in Extracts of Dried Leaves of *C. hindsii*

	solvent for extraction				
compound	H_2O[a]	50% methanol[b]	methanol[b]	50% ethanol[b]	ethanol[b]
1	2.09 ± 0.62[c]	2.74 ± 0.63	0.49 ± 0.07	2.73 ± 0.05	nd[d]
2	1.19 ± 0.71	2.37 ± 0.26	1.10 ± 0.12	1.59 ± 0.44	0.35 ± 0.07
3	6.07 ± 0.86	10.63 ± 0.74	6.60 ± 0.49	9.95 ± 0.61	1.50 ± 0.19
4	2.66 ± 0.39	1.70 ± 0.48	0.49 ± 0.21	1.03 ± 0.14	nd
5	0.85 ± 0.22	0.70 ± 0.17	0.26 ± 0.05	0.59 ± 0.03	nd
6	9.37 ± 0.53	13.82 ± 0.53	4.84 ± 0.45	10.10 ± 0.98	0.43 ± 0.12
7	3.38 ± 0.25	4.30 ± 0.71	2.25 ± 0.10	2.65 ± 0.61	nd
8	3.80 ± 0.11	5.87 ± 0.54	1.27 ± 0.50	4.42 ± 0.72	nd

[a] Sample was extracted with boiling water for 5 min. [b] Extracted at 50 °C for 1 h. [c] Amount of each compound was determined by HPLC analysis as described under Materials and Methods (mg/g of dried leaves). Each value is expressed as mean ± SD ($n = 3$). [d] Not detected.

(H-8*) and 5.83 (H-7*); **8**, δ 4.33 (H-8*) and 5.84 (H-7*), and 4.41 (H-8**) and 5.74 (H-7**)]. The ^{13}C NMR spectrum of each compound showed 54 carbon signals including characteristic signals of 6 carbonyl carbons [**7**, δ 168.6, 172.7, 172.9, 173.0, 173.1, and 173.6; **8**, δ 168.1, 172.2, 172.6 (2C), 173.1, and 173.5], a set of olefinic carbons (**7**, δ 114.1 and 148.0; **8**, δ 116.1 and 143.7), and 4 aliphatic carbons (**7**, δ 58.0 and 88.4, 58.3 and 88.4; **8**; δ 57.7 and 88.2, 58.1 and 88.3). The structural assignments of **7** and **8** were supported by HMBC analyses. **7**: the one methine proton signal at δ 4.45 (H-8*) gave cross-peaks with carbon signals at δ 126.9 (C-1′), 149.0 (C-3′), and 134.1 (C-1*); the other methine proton at δ 4.23 (H-8**) gave cross-peaks with carbon signals at δ 125.7 (C-1*′), 148.7 (C-3*′), 133.8 (C-1**); the proton signal at δ 5.83 (H-7*) gave cross-peaks with carbon signals at δ 113.6 (C-2*), 118.4 (C-6*), 126.2 (C-2′), 149.0 (C-3′), and 173.0 (C-9*); and the proton signal at δ 5.70 (H-7**) gave cross-peaks with carbon signals at δ 113.8 (C-2**), 118.6 (C-6**), 125.8 (C-2*′), 148.7 (C-3*′), and 173.1 (C-9**). **8**: the one methine proton signal at δ 4.33 (H-8*) gave cross-peaks with carbon signals at δ 124.5 (C-1), 148.9 (C-3), and 133.6 (C-1*), the other methine proton at δ 4.41 (H-8**) gave cross-peaks with carbon signals at δ 126.6 (C-1′), 148.8 (C-3′), and 134.0 (C-1**); the proton signal at δ 5.84 (H-7*) gave cross-peaks with carbon signals at δ 113.5 (C-2*), 118.1 (C-6*), 126.4 (C-2), 148.9 (C-3), and 172.2 (C-9*); and the proton signal at δ 5.74 (H-7**) gave cross-peaks with carbon signals at δ 113.4 (C-2**), 118.6 (C-6**), 126.1 (C-2′), 148.8 (C-3′), and 173.1 (C-9**). Furthermore, these NMR data were compared with those of compounds **3**, **5**, and **6**. The results indicate that **7** and **8** are both trimers of rosmarinic acid: in compound **7**, the conjugated olefinic linkage of the caffeic acid moiety of the first rosmarinic acid is attached to the benzene ring of the 3,4-dihydrophenyl lactic acid moiety of the second rosmarinic acid, and olefinic linkage of caffeic acid moiety of the third rosmarinic acid is attached to the benzene ring of the 3,4-dihydrophenyl lactic acid moiety of the first rosmarinic acid; and in compound **8**, the conjugated olefinic linkage of the caffeic acid moiety of the first rosmarinic acid is attached to the benzene ring of the caffeic acid moiety of the second rosmarinic acid, and olefinic linkage of caffeic acid moiety of the third rosmarinic acid is attached to the benzene ring of the 3,4-dihydrophenyl lactic acid moiety of the second rosmarinic acid. Thus, the structures were determined to be 4-[2-[3-(3,4-dihydroxyphenyl)-1-oxo-2-propenoxy]-2-carboxy-1-ethyl]-2-(3,4-dihydroxyphenyl)-2,3-dihydro-7-hydroxybenzofuran-3-carboxylic acid 1-carboxy-2-[2-(3,4-dihydroxyphenyl)-2,3-dihydro-7-hydroxybenzofuran-3-carboxylic acid 1-carboxy-2-(3,4-dihydroxyphenyl)ethyl ester]-ethyl ester (**7**) and 4-[3-[1-carboxy-2-[2-(3,4-dihydroxyphenyl)-2,3-dihydro-7-hydroxybenzofuran-3-carboxylic acid 1-carboxy-2-(3,4-dihydroxyphenyl)ethyl ester]ethoxy]-3-oxo-1-propenyl]-2-(3,4-dihydroxyphenyl)-2,3-dihydro-7-hydroxybenzofuran-3-carboxylic acid 1-carboxy-2-(3,4-dihydroxyphenyl)ethyl ester (**8**), respectively. The stereochemistry of compounds **7** and **8** at the positions of C-7*, C-8*, C-7**, and C-8** of the dihydrobenzofuran moieties could not be resolved.

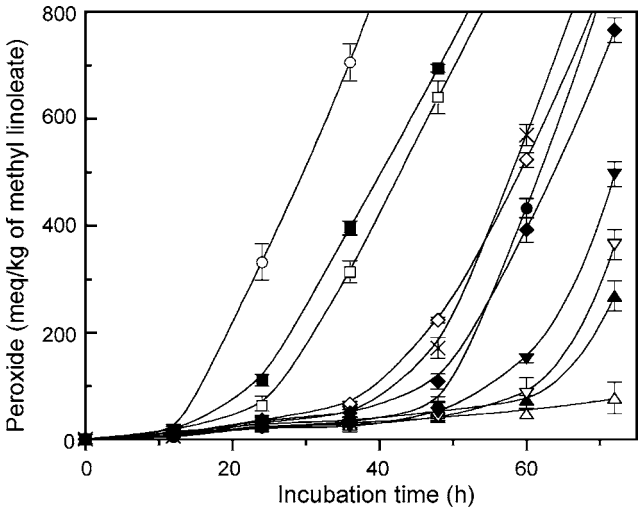

Figure 3. Effect of compounds **1**–**8** on the autoxidation of methyl linoleate in bulk phase: (○) no addition; (□) **1**; (■) **2**; (△) **3**; (▲) **4**; (▽) **5**; (▼) **6**; (◇) **7**; (◆) **8**; (●) α-tocopherol; (×) quercetin. Each value is expressed as mean ± standard deviation of three different experiments.

Compounds **1**−**8** in the dried leaves of *C. hindsii* were extracted with different solvent systems, and the amount of each compound was quantified by RP-HPLC (**Table 3**). The solvents for extraction affected the yields of compounds **1**−**8**. The highest yield was obtained in the use of 50% methanol as the solvent. On the other hand, the ethanol extract gave only small amounts of these compounds. In the 50% methanol extract, the most abundant compounds were rosmarinic acid (**3**) and lithospermic acid B (**6**) in addition to relatively large amounts of rosmarinic acid trimers (**7** and **8**).

Antioxidative Activities of Compounds 1−**8.** Water-soluble phenolic acids, such as caffeic acid, rosmarinic acid, and their derivatives, lithospermic acid and lithospermic acid B, have been reported to possess various biological effects, including antioxidative activities (*23*, *32*). Therefore, we evaluated the antioxidative activities of compounds **1**−**8** because of the existence of phenolic groups in the molecules.

Figure 3 shows the inhibitory effect of compounds **1**−**8** during the autoxidation of methyl linoleate in bulk phase. Differences in antioxidative behavior of the isolated compounds were observed in their inhibition of methyl linoleate autoxidation. Rosmarinic acid (**3**) and its derivatives (**4**−**8**) showed high antioxidative activity, which was comparable to that of α-toco-

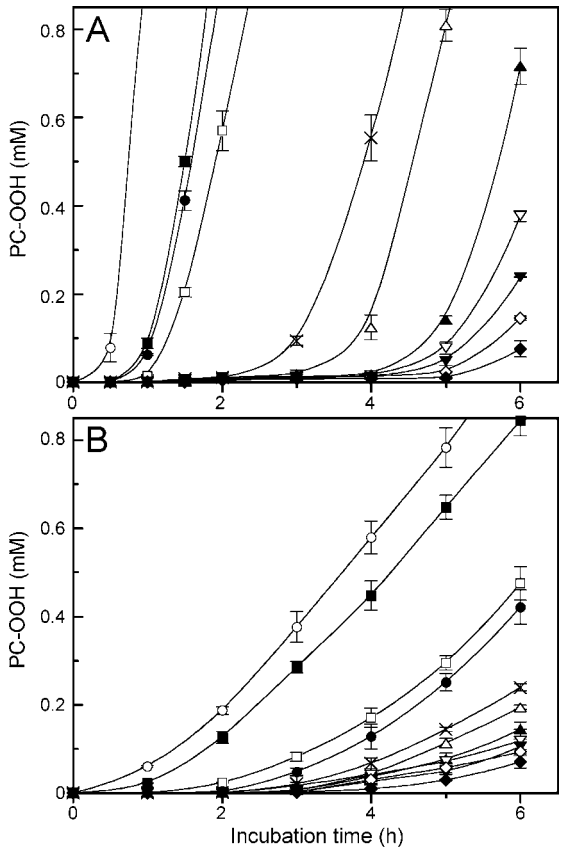

Figure 4. Effect of compounds **1**–**8** on the AAPH-induced (**A**) and AMVN-induced (**B**) peroxidation of soybean PC in liposomes: (○) no addition; (□) **1**; (■) **2**; (△) **3**; (▲) **4**; (▽) **5**; (▼) **6**; (◇) **7**; (◆) **8**; (●) α-tocopherol; (×) quercetin. Each value is expressed as mean ± standard deviation of three different experiments.

pherol and quercetin. However, two flavonol glycosides (**1** and **2**) exhibited less antioxidative activity. Among the rosmarinic acid compounds (**3**−**8**), the degree of polymerization affected their effectiveness of antioxidation. The results indicate that the antioxidative activity of the isolated compounds might be influenced by their solubility in the bulk oil.

The antioxidative activity of compounds **1**−**8** during the peroxidation of soybean PC liposomes induced by AAPH or AMVN was also evaluated (**Figure 4**). **Figure 4A** shows the effect of compounds **1**−**8** on the AAPH-induced PC peroxidation. The formation of PC-OOH was inhibited by the addition of compounds **1**−**8**, α-tocopherol, or quercetin. Quercetin and rosmarinic acid compounds (**3**−**8**) showed higher antioxidative activity compared with flavonoid glycosides (**1** and **2**) and α-tocopherol. The order of their inhibitory effect correlated with the number of phenolic hydroxyl groups in each molecule. The water-soluble radical generator, AAPH, produces peroxyl radicals in the aqueous phase, and the resulting peroxyl radicals can attack phospholipids on the membrane surface. Thus, compounds **3**−**8** and quercetin might be located near the membrane surface, where they would scavenge aqueous chain-initiating peroxyl radicals from AAPH. On the other hand, α-tocopherol could not suppress this liposomal peroxidation efficiently due to the existence of the inner lipid phase. When the peroxidation was started in the lipid phase, their antioxidative effects were slightly different (**Figure 4B**). The peroxidation was suppressed effectively by the addition of **1**, **3**−**8**, α-tocopherol, and quercetin. α-Tocopherol is a well-known lipid-soluble antioxidant. Thus, these antioxidants might be located on the surface of or inside the membranes, where they scavenge peroxyl radicals generated in the lipid phase. In the present liposomal systems, rosmarinic acid (**3**) and its derivatives (**4**−**8**) exhibited stronger antioxidative activity than that of α-tocopherol or flavonoid constantly.

This study shows that the dried leaves of *C. hindsii* contain large amounts of rosmarinic acid (**3**), lithospermic acid B (**6**), and lesser amounts of their derivatives (**4**, **5**, **7**, and **8**). These compounds could suppress the autoxidation of methyl linoleate in bulk phase and the radical-initiated peroxidation of soybean phosphatidylcholine in liposomes. Therefore, the extract of *C. hindsii* is expected to be as a source of natural antioxidants. Further studies are needed to understand the possible benefits of *C. hindsii* polyphenols to human health and food products.

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