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Isolation and Characterization of Some Antioxidative Compounds from the Rhizomes of Smaller Galanga (*Alpinia officinarum* Hance)

TRAM NGOC LY,† MAKOTO SHIMOYAMADA,‡ KOJI KATO,§ AND RYO YAMAUCHI*,‡

The United Graduate School of Agricultural Science, Department of Bioprocessing, and Department of Food Science, Faculty of Agriculture, Gifu University, 1-1 Yanagido, Gifu City, Gifu 501-1193, Japan

Antioxidative compounds were isolated from the methanol extract of fresh rhizome of smaller galanga (*Alpinia officinarum* Hance). Seven phenylpropanoids (**1**−**7**) were finally obtained by reversed-phase HPLC, and their structures were elucidated by MS and NMR analyses. They comprised the two known compounds, (*E*)-*p*-coumaryl alcohol γ-*O*-methyl ether (**1**) and (*E*)-*p*-coumaryl alcohol (**6**), and the five novel compounds, stereoisomers of (4*E*)-1,5-bis(4-hydroxyphenyl)-1-methoxy-2-(methoxymethyl)-4-pentene (**2a** and **2b**), stereoisomers of (4*E*)-1,5-bis(4-hydroxyphenyl)-1-ethoxy-2-(methoxymethyl)-4-pentene (**3a** and **3b**), (4*E*)-1,5-bis(4-hydroxyphenyl)-1-[(2*E*)-3-(4-acetoxyphenyl)-2-propenoxy]-2-(methoxymethyl)-4-pentene (**4**), (4*E*)-1,5-bis(4-hydroxyphenyl)-2-(methoxymethyl)-4-penten-1-ol (**5**), and (4*E*)-1,5-bis(4-hydroxyphenyl)-2-(hydroxymethyl)-4-penten-1-ol (**7**). Compounds **1**−**7** were detected for the first time as constituents of galanga rhizomes and exhibited antioxidative activities against the autoxidation of methyl linoleate in bulk phase.

KEYWORDS: Antioxidant; phenylpropanoid; *p*-coumaryl alcohol; *p*-coumaryl methyl ether; galanga; *Alpinia officinarum* Hance; Zingiberaceae

INTRODUCTION

The oxidative deterioration of lipids is a great concern in the shelf life of foods. Spices and herbs provide foods with flavors and food-preserving power, including antiseptic and antioxidative activities. Natural antioxidants of spices and herbs are generally classified as vitamins, phenolic compounds including flavonoids and phenolic acids, and volatile compounds (*1*). Extracts of various spices and herbs possess antioxidative activities, and many antioxidative compounds have been identified (*2*−*13*). In the ginger family (Zingiberaceae), extracts from various species of gingers in tropical countries have been demonstrated to have strong antioxidative activities that could replace α-tocopherol (*3*). The rhizome of a popular ginger species (*Zingiber officinale* Roscoe) is well-known to have potent antioxidative activity, and some antioxidative compounds have been isolated: gingerol and shogaol (*2*), gingerol-related compounds and diarylheptanoids (*6*), and glycosides related to gingerdiol (*9*). Curcuminoids are other antioxidative compounds in some species of the ginger family (*4*, *5*, *7*).

Smaller galanga (*Alpinia officinarum* Hance) is a pungent and aromatic rhizome, which is a member of the ginger family. The rhizome is cultivated in Vietnam and southern China because of its use as a spice and as a traditional medicine (*14*). We previously reported that the rhizomes of smaller galanga contained 1,8-cineole, eugenol, chavicol, and other phenylpropanoids as the major components of its essential oil (*15*). In addition, we isolated and characterized some glycosides in fresh rhizomes of smaller galanga (*16*). The main glycosides were β-glycosides of chavicol and 1,2-dihydroxy-4-allylbenzene, which contained one or two phenolic hydroxyl groups in the molecule and might be expected to act as antioxidants after elimination of their sugar moieties. Cheah and Hasim (*17*) reported that the extract from rhizomes of greater galanga (*Alpinia galanga* Willd.) was effective in inhibiting lipid peroxidation in raw and cooked beef, although they did not isolate active components. Therefore, some antioxidative compounds could be present in smaller galanga. This study was designed to isolate and characterize antioxidative compounds in fresh rhizomes of smaller galanga.

MATERIALS AND METHODS

Plant Material. Fresh rhizomes of smaller galanga (*A. officinarum* Hance) were purchased at a local market in Hanoi, Vietnam, and botanically authenticated at the National Center for Scientific Research Technology, Vietnam. The rhizomes were then stored at −20 °C until analysis.

Reagents. Methyl linoleate (Tokyo Kasei Kogyo Co., Tokyo, Japan) was purified by silica gel column chromatography to be peroxide-free (*18*). Methyl linoleate monohydroperoxide was isolated from autoxi-

* Author to whom correspondence should be addressee (telephone/fax +81-58-293-2930; e-mail yamautir@cc.gifu-u.ac.jp).
† The United Graduate School of Agricultural Science.
‡ Department of Bioprocessing.
§ Department of Food Science.

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dized methyl linoleate by silica gel column chromatography (*19*). *RRR*-α-Tocopherol from Sigma Chemical Co. (St. Louis, MO) was purified by high-performance liquid chromatography (HPLC) before use (*20*).

Apparatus. Specific rotations were determined with an SEPA-300 polarimeter (Horiba, Ltd., Kyoto, Japan). Ultraviolet (UV) spectra were measured with a Jasco Ubest-30 UV−vis spectrophotometer (Japan Spectroscopic Co., Tokyo, Japan). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded with an INOVA 400 FT-NMR spectrometer (Varian, Palo Alto, CA) with CD_3OD as the solvent and tetramethylsilane as the internal standard. ^{1}H NMR was performed at 400.0 MHz, and the ^{1}H−^{1}H chemical shift-correlated (COSY) NMR technique was employed to assign ^{1}H shifts and couplings. ^{13}C NMR was at 100.6 MHz with proton decoupling. ^{1}H detected heteronuclear multiple-bond correlation (HMBC) spectroscopy was used to assign correlations between ^{1}H and ^{13}C signals. Nuclear Overhauser and exchange spectroscopy (NOESY) was used to establish the absolute configuration. High-resolution electron-impact mass spectrometry (HR-EIMS) and electron-impact mass spectrometry (EIMS) were performed on a JMS-GC Mate II instrument (JEOL, Tokyo, Japan) operated at 70 eV. Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) mass spectra were obtained with an LCMS-QP8000α instrument (Shimadzu Co., Kyoto, Japan) equipped with ESI and APCI sources, respectively. Sample was delivered into the ion source using methanol/water (7:3, v/v) containing 0.15% acetic acid at 0.1 mL/min.

Extraction and Isolation Procedures. Fresh rhizomes of galanga (4.00 kg) were chopped and homogenized in methanol and successively extracted three times with hot methanol for 3 h. After the solvent was removed, the methanol extract (260 g) was subjected to chromatography on a 20 × 6.0 cm Amberlite XAD-2 resin column. The column was rinsed with distilled water (3 L) to eliminate water-soluble compounds, and the retained material was eluted with methanol (3 L). The methanol eluate was concentrated to dryness in vacuo. The residue (40.0 g) was then suspended in water (250 mL) and partitioned with chloroform (400 mL) to afford chloroform-soluble (10.2 g) and water-soluble fractions (29.6 g), respectively.

The chloroform-soluble fraction (10.2 g) was subjected to chromatography on a 22 × 4 cm BW-820MH, 70−200 mesh silica gel column (Fuji Silysia Chemical Ltd., Kasugai, Japan). The column was sequentially eluted by increasing methanol concentration in mixtures of chloroform and methanol. Each fraction was checked by thin-layer chromatography (TLC) on silica gel 60, 0.25 mm thickness (Merck, Darmstadt, Germany), developed with chloroform/methanol/acetic acid (90:10:1, v/v/v). Spots on the TLC plate were visualized by charring after spraying with 50% H_2SO_4 or by spraying with a mixture of 0.2% $FeCl_3$ and 0.5% *o*-phenanthroline in ethanol. Antioxidative compounds were eluted with chloroform/methanol (95:5, v/v), and fractions with the same TLC patterns were pooled to give fractions I (1.27 g), II (0.62 g), and III (0.45 g). Fractions I−III were further purified by HPLC. Reversed-phase HPLC was done with a 250 × 10 mm Hydrosphere C18 column (YMC Co., Ltd., Kyoto, Japan) developed with acetonitrile/water (35:65, v/v; for fractions I and II) or acetonitrile/water (30:70, v/v; for fraction III) at a flow rate of 5.0 mL/min. The eluate was monitored by an absorbance at 210 nm. Compound **1** (320.0 mg) was isolated from fraction I, compounds **2a** (71.0 mg), **2b** (28.0 mg), **3a** (11.7 mg), **3b** (3.5 mg), and **4** (21.0 mg) were from fraction II, and compound **5** (14.0 mg) was from fraction III.

The water-soluble fraction (29.6 g) was subjected to chromatography on an 18 × 4.0 cm ODS-A 120-S 150 silica gel column (YMC Co., Ltd., Kyoto, Japan) with stepwise elution of methanol in water (10, 20, 30, 40, 50, and 100%; each 900 mL). The 20% methanol eluate (1.16 g) and 30% methanol eluate (2.17 g), which had antioxidative compounds, were further subjected to chromatography on an 18 × 3.4 cm BW-820MH, 70−200 mesh silica gel column, respectively. The column was sequentially eluted by increasing the methanol concentration in mixtures of chloroform/methanol. Each fraction was checked by TLC on silica gel 60 developed with chloroform/methanol/water (65:25:4, v/v/v). Fraction IV (390 mg) was obtained from the 20% methanol eluate with elution of chloroform/methanol (97:3, v/v), whereas fraction V (57.6 mg) was obtained from the 30% methanol eluate with elution of chloroform/methanol (95:5, v/v). Fractions IV and V were further purified by HPLC. Reversed-phase HPLC was done with a 250 × 10 mm Hydrosphere C18 column developed with acetonitrile/water (15:85, v/v; for fraction IV) or acetonitrile/water (22:78, v/v; for fraction V) at a flow rate of 5.0 mL/min. Compound **6** (350.0 mg) was isolated from fraction IV, and compound **7** (16.0 mg) was from fraction V.

Structures of Compounds 1—7. *(E)-p-Coumaryl alcohol γ-O-methyl ether [4-[(1E)-3-methoxy-1-propenyl]phenol, **1**]:* white amorphous solid; UV (methanol) λ_{max} (log ϵ) 261 nm (4.35); HR-EIMS, *m/z* 164.0874 (M^+), calcd for $C_{10}H_{12}O_2$, 164.0837; EIMS, *m/z* 164 (M^+, 26%), 132 ($[M - CH_3OH]^+$, 100), 103 (36), and 77 (24); APCIMS (positive), *m/z* 133.05 ($[M - OCH_3]^+$, 100%); APCIMS (negative), *m/z* 162.95 ($[M - H]^-$, 100%) and 147.45 ($[M - OH]^-$, 40); ^{1}H NMR (CD_3OD) δ 3.34 (s, 3H, 3-OC*H*$_3$), 4.03 (d, J = 6.2 Hz, 2H, H-3), 6.10 (td, J = 6.2, 15.8 Hz, 1H, H-2), 6.52 (d, J = 16.1 Hz, 1H, H-1), 6.74 (d, J = 8.8 Hz, 2H, H-3′, H-5′), and 7.25 (d, J = 8.4 Hz, 2H, H-2′, H-6′).

*Stereoisomers of (4E)-1,5-bis(4-hydroxyphenyl)-1-methoxy-2-(methoxymethyl)-4-pentene (**2a** and **2b**):* **2a**: pale yellow wax; $[\alpha]^{27}_D$ −0.57 (*c* 0.52, methanol); UV (methanol) λ_{max} (log ϵ) 261 nm (4.42); HR-EIMS, *m/z* 328.1735 (M^+), calcd for $C_{20}H_{24}O_4$, 328.1675; EIMS, *m/z* 328 (M^+, 24%), 296 ($[M - CH_3OH]^+$, 76), 264 ($[M - (CH_3OH)_2]^+$, 31), 251 (100), 189 (19), 164 (21), 137 (94), 133 (87), 121 (34), 107 (82), and 77 (20); ESIMS (positive), *m/z* 351.10 ($[M + Na]^+$, 100%); ESIMS (negative), *m/z* 327.05 ($[M - H]^-$, 100%); ^{1}H NMR (CD_3OD) δ 2.02 (m, 2H, H-2, H-3a), 2.09 (m, 1H, H-3b), 3.14 (s, 3H, 1-OC*H*$_3$), 3.31 (s, 3H, 6-OC*H*$_3$), 3.40 (dd, J = 4.0, 9.2 Hz, 1H, H-6a), 3.53 (dd, J = 5.1, 9.2 Hz, 1H, H-6b), 4.10 (d, J = 7.3 Hz, 1H, H-1), 5.87 (td, J = 7.3, 15.7 Hz, 1H, H-4), 6.14 (d, J = 15.7 Hz, 1H, H-5), 6.70 (d, J = 8.4 Hz, 2H, H-3″, H-5″), 6.80 (d, J = 8.4 Hz, 2H, H-3′, H-5′), 7.10 (d, J = 8.4 Hz, 2H, H-2′, H-6′), and 7.12 (d, J = 8.4 Hz, 2H, H-2″, H-6″). **2b**: yellow wax; $[\alpha]^{27}_D$ +1.0 (*c* 0.58, methanol); UV (methanol) λ_{max} (log ϵ) 261 nm (4.39); HR-EIMS, *m/z* 328.1625 (M^+), calcd for $C_{20}H_{24}O_4$ 328.1675; EIMS, *m/z* 328 (M^+, 8%), 296 ($[M - CH_3OH]^+$, 39), 264 ($[M - (CH_3OH)_2]^+$, 29), 251 (100), 189 (10), 164 (23), 137 (99), 133 (69), 121 (15), 107 (59), and 77 (17); ESIMS (positive), *m/z* 351.10 ($[M + Na]^+$, 100%); ESIMS (negative), *m/z* 327.05 ($[M - H]^-$, 100%); ^{1}H NMR (CD_3OD) δ 1.90 (m, 1H, H-2), 2.30 (ddd, J = 5.5, 8.4, 16.8 Hz, 1H, H-3a), 2.46 (m, 1H, H-3b), 3.00 (dd, J = 5.1, 9.2 Hz, 1H, H-6a), 3.16 (s, 3H, 1-OC*H*$_3$), 3.18 (s, 3H, 6-OC*H*$_3$), 3.26 (dd, J = 4.8, 9.2 Hz, 1H, H-6b), 4.10 (d, J = 7.3 Hz, 1H, H-1), 5.99 (ddd, J = 6.6, 8.4, 15.7 Hz, 1H, H-4), 6.28 (d, J = 15.7 Hz, 1H, H-5), 6.70 (d, J = 8.8 Hz, 2H, H-3″, H-5″), 6.78 (d, J = 8.4 Hz, 2H, H-3′, H-5′), 7.10 (d, J = 8.4 Hz, 2H, H-2′, H-6′), and 7.16 (d, J = 8.4 Hz, 2H, H-2″, H-6″).

*Stereoisomers of (4E)-1,5-bis(4-hydroxyphenyl)-1-ethoxy-2-(methoxymethyl)-4-pentenes (**3a** and **3b**):* **3a**: yellow wax; $[\alpha]^{27}_D$ +2.9 (*c* 0.17, methanol); UV (methanol) λ_{max} (log ϵ) 261 nm (4.37); HR-EIMS, *m/z* 342.1797 (M^+), calcd for $C_{21}H_{26}O_4$, 342.1831; EIMS, *m/z* 342 (M^+, 13%), 310 ($[M - CH_3OH]^+$, 14), 296 ($[M - C_2H_5OH]^+$, 33), 264 (27), 251 (100), 189 (9), 164 (21), 137 (18), 133 (70), 123 (81), 107 (53), and 77 (24); ESIMS (positive), *m/z* 365.10 ($[M + Na]^+$, 100%); ESIMS (negative), *m/z* 340.95 ($[M - H]^-$, 100%); ^{1}H NMR (CD_3OD) δ 1.13 (t, J = 7.0, 3H, 1-OCH$_2$C*H*$_3$), 1.98 (m, 2H, H-2, H-3a), 2.07 (m, 1H, H-3b), 3.25 (qd, J = 7.0, 7.3 Hz, 1H, 1-OC*H*$_2$CH$_3$), 3.31 (s, 3H, 6-OC*H*$_3$), 3.34 (qd, J = 7.0, 7.3 Hz, 1H, 1-OC*H*$_2$CH$_3$), 3.42 (dd, J = 4.0, 9.5 Hz, 1H, H-6a), 3.55 (dd, J = 5.5, 9.5 Hz, 1H, H-6b), 4.21 (d, J = 7.0 Hz, 1H, H-1), 5.87 (td, J = 7.3, 15.7 Hz, 1H, H-4), 6.14 (d, J = 15.7 Hz, 1H, H-5), 6.68 (d, J = 8.8 Hz, 2H, H-3″, H-5″), 6.78 (d, J = 8.8 Hz, 2H, H-3′, H-5′), 7.10 (d, J = 8.4 Hz, 2H, H-2′, H-6′), and 7.12 (d, J = 8.8 Hz, 2H, H-2″, H-6″). **3b**: pale yellow wax; $[\alpha]^{27}_D$ +3.3 (*c* 0.06, methanol); UV (methanol) λ_{max} (log ϵ) 261 nm (4.32); HR-EIMS, *m/z* 342.1769 (M^+), calcd for $C_{21}H_{26}O_4$, 342.1831; EIMS, *m/z* 342 (M^+, 5%), 310 ($[M - CH_3OH]^+$, 3), 296 ($[M - C_2H_5OH]^+$, 50), 264 (20), 251 (100), 189 (11), 164 (12), 151 (100), 133 (53), 123 (75), 107 (63), and 77 (20); ESIMS (positive), *m/z* 365.15 ($[M + Na]^+$, 100%); ESIMS (negative), *m/z* 341.05 ($[M - H]^-$, 100%); ^{1}H NMR (CD_3OD) δ 1.15 (t, J = 7.0 Hz, 3H, 1-OCH$_2$C*H*$_3$), 1.88 (m, 1H, H-2), 2.31 (m, 1H, H-3a), 2.47 (m, 1H, H-3b), 3.00 (dd, J = 5.5, 9.5 Hz, 1H, H-6a), 3.19 (s, 3H, 6-OC*H*$_3$), 3.25 (qd, J = 7.3, 9.2 Hz, 1H, 1-OC*H*$_2$CH$_3$), 3.28 (dd, J = 4.4, 9.5 Hz, 1H, H-6b), 3.37 (qd, J = 7.3, 9.2 Hz, 1H, 1-OC*H*$_2$CH$_3$), 4.22 (d, J = 7.3 Hz, 1H, H-1), 6.01 (ddd, J

= 6.2, 8.1, 15.7 Hz, 1H, H-4), 6.28 (d, J = 15.7 Hz, 1H, H-5), 6.69 (d, J = 8.4 Hz, 2H, H-3″, H-5″), 6.76 (d, J = 8.4 Hz, 2H, H-3′, H-5′), 7.11 (d, J = 8.4 Hz, 2H, H-2′, H-6′), and 7.16 (d, J = 8.8 Hz, 2H, H-2″, H-6″).

*(4E)-1,5-Bis(4-hydroxyphenyl)-1-[(2E)-3-(4-acetoxyphenyl)-2-propenoxy]-2-(methoxymethyl)-4-pentene (**4**):* colorless wax; $[\alpha]^{27}_D$ +0.58 (*c* 0.35, methanol); UV (methanol) λ_{max} (log ϵ) 257 nm (4.67); HR-EIMS, *m/z* 488.1809 (M^+), calcd for $C_{30}H_{32}O_6$, 488.2199; EIMS, *m/z* 488 (M^+, 4%), 338 (6%), 323 (5%), 306 (14), 293 (11), 264 (38), 251 (28), 189 (9), 175 (14), 164 (23), 133 (100), 107 (99), and 77 (22); ESIMS (positive), *m/z* 511.10 ($[M + Na]^+$, 100%); ESIMS (negative), *m/z* 487.05 ($[M - H]^-$, 10%) and 445.00 ($[M - CH_3CO]^-$, 100); ^{1}H NMR (CD_3OD) δ 1.98 (m, 1/5H, H-2), 2.05 (m, 8/5H, H-2, H-3a), 2.11 (m, 4/5H, H-3b), 2.26 (s, 3H, 4‴-OCOCH_3), 2.39 (m, 1/5H, H-3a), 2.51 (m, 1/5H, H-3b), 3.03 (dd, J = 5.6, 9.3 Hz, 1/5H, H-6a), 3.18 (s, 3/5H, 6-OCH_3), 3.26 (dd, J = 4.8, 9.2 Hz, 1/5H, H-6b), 3.31 (s, 12/5H, 6-OCH_3), 3.44 (dd, J = 4.0, 9.5 Hz, 4/5H, H-6a), 3.59 (dd, J = 5.5, 9.5 Hz, 4/5H, H-6b), 3.868 and 3.871 (dd, J = 6.6, 12.8 Hz, 1H, H-9‴a), 4.019 and 4.022 (dd, J = 5.5, 12.8 Hz, 1H, H-9‴b), 4.34 (d, J = 7.7 Hz, 1/5H, H-1), 4.37 (d, J = 6.6 Hz, 4/5H, H-1), 5.90 (td, J = 7.3, 15.7 Hz, 4/5H, H-4), 6.05 (m, 1/5H, H-4), 6.16 (d, J = 15.7 Hz, 4/5H, H-5), 6.24 (ddd, J = 5.5, 6.6, 16.1 Hz, 1H, H-8‴), 6.30 (d, J = 16.5 Hz, 1/5H, H-5), 6.51 (d, J = 15.7 Hz, 1H, H-7‴), 6.67 (d, J = 8.8 Hz, 2H, H-3″, H-5″), 6.81 (d, J = 8.8 Hz, 2H, H-3′, H-5′), 7.01 (d, J = 8.4 Hz, 2H, H-3‴, H-5‴), 7.09 (d, J = 8.4 Hz, 2H, H-2″, H-6″), 7.14 (d, J = 8.4 Hz, 2H, H-2′, H-6′), 7.36 (d, J = 8.4 Hz, 2H, H-2‴, H-6‴).

*(4E)-1,5-Bis(4-hydroxyphenyl)-2-(methoxymethyl)-4-penten-1-ol (**5**):* colorless wax; $[\alpha]^{27}_D$ +4.3 (*c* 0.12, methanol); UV (methanol) λ_{max} (log ϵ) 261 nm (4.44); HR-EIMS, *m/z* 314.1516 (M^+), calcd for $C_{19}H_{22}O_4$, 314.1518; EIMS, *m/z* 314 (M^+, 21%), 296 ($[M - H_2O]^+$, 20), 282 ($[M - CH_3OH]^+$, 34), 264 ($[M - (H_2O + CH_3OH)]^+$, 45), 251 (74), 199 (25), 180 (36), 164 (33), 160 (35), 145 (39), 133 (100), 123 (61), 121 (92), 107 (97), and 77 (36); ESIMS (positive), *m/z* 336.85 ($[M + Na]^+$, 100%); ESIMS (negative), *m/z* 313.00 ($[M - H]^-$, 100%); ^{1}H NMR (CD_3OD) δ 1.92 (m, 1/5H, H-2), 1.99 (m, 8/5H, H-2, H-3a), 2.13 (m, 4/5H, H-3b), 2.25 (m, 1/5H, H-3a), 2.41 (m, 1/5H, H-3b), 3.10 (dd, J = 4.8, 9.2 Hz, 1/5H, H-6a), 3.23 (s, 3/5H, 6-OCH_3), 3.33 (s, 12/5H, 6-OCH_3), 3.34 (dd, J = 5.6, 9.6 Hz, 1/5H, H-6b), 3.43 (dd, J = 4.8, 9.5 Hz, 4/5H, H-6a), 3.56 (dd, J = 4.8, 9.5 Hz, 4/5H, H-6b), 4.60 (d, J = 6.6 Hz, 4/5H, H-1), 4.66 (d, J = 6.6 Hz, 1/5H, H-1), 5.90 (ddd, J = 6.6, 8.1, 15.7 Hz, 4/5H, H-4), 5.99 (ddd, J = 6.8, 8.8, 16.0 Hz, 1/5H, H-4), 6.17 (d, J = 15.7 Hz, 4/5H, H-5), 6.28 (d, J = 15.7 Hz, 1/5H, H-5), 6.68 (d, J = 8.4 Hz, 2H, H-3″, H-5″), 6.77 (d, J = 8.4 Hz, 2H, H-3′, H-5′), 7.13 (d, J = 8.4 Hz, 2H, H-2″, H-6″), and 7.16 (d, J = 8.8 Hz, 2H, H-2′, H-6′).

*(E)-p-Coumaryl alcohol (4-[(1E)-3-hydroxy-1-propenyl]phenol, **6**):* white amorphous solid; UV (methanol) λ_{max} (log ϵ) 260 nm (4.32); HR-EIMS, *m/z* 150.0698 (M^+), calcd for $C_9H_{10}O_2$, 150.0681; EIMS, *m/z* 150 (M^+, 50%), 132 ($[M - H_2O]^+$, 19), 107 (97), 103 (23), 94 (43), and 77 (24); APCIMS (negative), *m/z* 149.00 ($[M - H]^-$, 100%); ^{1}H NMR (CD_3OD) δ 4.18 (dd, J = 1.1, 5.9 Hz, 2H, H-3), 6.16 (td, J = 5.9, 15.8 Hz, 1H, H-2), 6.50 (d, J = 15.8 Hz, 1H, H-1), 6.73 (d, J = 8.4 Hz, 2H, H-3′, H-5′), and 7.24 (d, J = 8.8 Hz, 2H, H-2′, H-6′).

*(4E)-1,5-Bis(4-hydroxyphenyl)-2-(hydroxymethyl)-4-penten-1-ol (**7**):* colorless wax; $[\alpha]^{27}_D$ −13.2 (*c* 0.19, methanol); UV (methanol) λ_{max} (log ϵ) 261 nm (4.37); HR-EIMS, *m/z* 300.1464 (M^+), calcd for $C_{18}H_{20}O_4$, 300.1362; EIMS, *m/z* 300 (M^+, 5%), 282 ($[M - H_2O]^+$, 15), 264 ($[M - (H_2O)_2]^+$, 13), 252 (100), 199 (33), 166 (11), 158 (43), 145 (88), 133 (40), 123 (18), 107 (83), and 77 (20); ESIMS (positive), *m/z* 322.95 ($[M + Na]^+$, 100%); ESIMS (negative), *m/z* 298.90 ($[M - H]^-$, 100%); ^{1}H NMR (CD_3OD) δ 1.91 (m, 1H, H-2), 2.00 (ddd, J = 7.0, 8.1, 15.0 Hz, 1H, H-3a), 2.12 (ddd, J = 7.0, 8.4, 14.0 Hz, 1H, H-3b), 3.65 (dd, J = 5.5, 11.0 Hz, 1H, H-6a), 3.78 (dd, J = 4.8, 11.0 Hz, 1H, H-6b), 4.62 (d, J = 7.3 Hz, 1H, H-1), 5.91 (ddd, J = 7.0, 7.7, 15.7 Hz, 1H, H-4), 6.20 (d, J = 15.7 Hz, 1H, H-5), 6.68 (d, J = 8.8 Hz, 2H, H-3″, H-5″), 6.77 (d, J = 8.8 Hz, 2H, H-3′, H-5′), 7.13 (d, J = 8.8 Hz, 2H, H-2″, H-6″), 7.19 (d, J = 8.4 Hz, 2H, H-2′, H-6′).

Antioxidative Activity. The antioxidative activity of each isolated compounds was measured by its inhibition of methyl linoleate autoxidation in bulk phase (*21*). Methyl linoleate (294 mg, 1.0 mmol) containing the isolated compound (each 0.25 μmol; 0.025 mol %, based on methyl linoleate) or α-tocopherol (0.025 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 24 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 the iodometric method (*18*). Methyl linoleate monohydroperoxide was used as the standard peroxide.

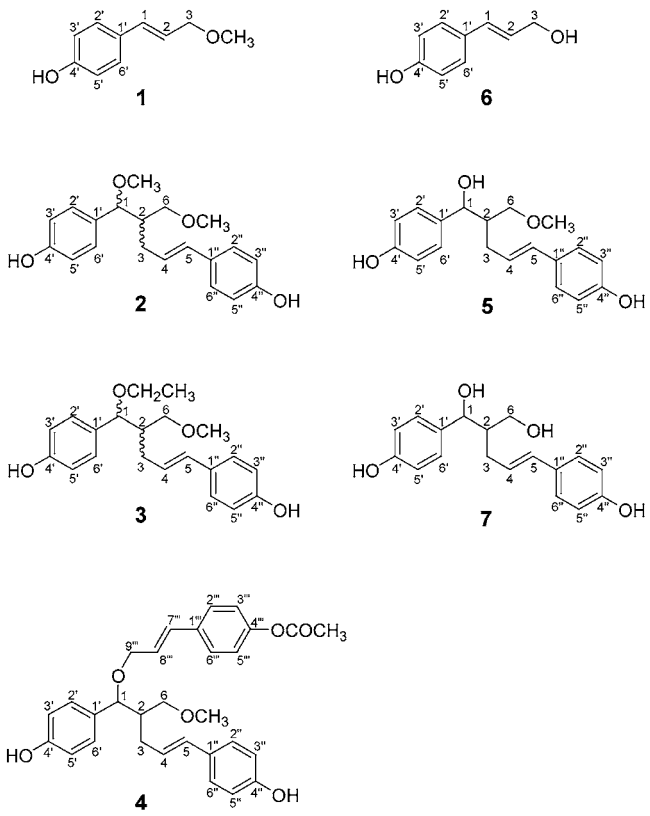

Figure 1. Structures of (*E*)-*p*-coumaryl alcohol γ-*O*-methyl ether (**1**), (4*E*)-1,5-bis(4-hydroxyphenyl)-1-methoxy-2-(methoxymethyl)-4-pentene (**2**), (4*E*)-1,5-bis(4-hydroxyphenyl)-1-ethoxy-2-(methoxymethyl)-4-pentene (**3**), (4*E*)-1,5-bis(4-hydroxyphenyl)-1-[(2*E*)-3-(4-acetoxyphenyl)-2-propenoxy]-2-(methoxymethyl)-4-pentene (**4**), (4*E*)-1,5-bis(4-hydroxyphenyl)-2-(methoxymethyl)-4-penten-1-ol (**5**), (*E*)-*p*-coumaryl alcohol (**6**), and (4*E*)-1,5-bis(4-hydroxyphenyl)-2-(hydroxymethyl)-4-penten-1-ol (**7**) isolated from fresh rhizomes of smaller galanga (*A. officinarum* Hance).

RESULTS AND DISCUSSION

Structures of Isolated Compounds. Methanol extract from galanga rhizomes was subjected to Amberlite XAD-2 column chromatography followed by silica gel column chromatography. Finally, the fractions containing antioxidative compounds were purified by preparative reversed-phase HPLC to obtain compounds **1**−**7**. The structures of compounds **1**−**7** are characterized as follows (**Figure 1**).

Compound **1** was obtained as a white amorphous solid (320.0 mg) and identified to be (*E*)-*p*-coumaryl alcohol γ-*O*-methyl ether [4-[(1*E*)-3-methoxy-1-propenyl]phenol] by comparison of its ^{1}H and ^{13}C NMR data with those in the literature (*22*). This compound has been detected in the epicuticular wax of Gala apple fruits (*Malus domestica* Borkh. Rosaceae).

Compounds **2a** and **2b** were obtained as a pale yellow wax (71.0 mg) and a yellow wax (28.0 mg), respectively. HR-EIMS of **2a** and **2b** gave the same molecular formula of $C_{20}H_{24}O_4$. Their ^{1}H and ^{13}C NMR spectra were very similar to one another. However, differences in chemical shift and coupling constant

Table 1. ^{13}C NMR Data for Compounds **1**–**7**[a]

	compound								
carbon	**1**	**2a**[b]	**2b**	**3a**	**3b**	**4**	**5**	**6**	**7**
1	134.2	84.6	84.9	82.6	82.8	81.9 (82.0)[c]	75.4 (74.7)	131.9	76.4
1-OCH_3		56.8	57.0						
1-OCH_2CH_3				65.0	65.2				
1-OCH_2CH_3				15.6	15.6				
2	123.3	46.9	47.5	47.0	47.6	47.0 (47.4)	47.3 (47.9)	126.6	49.0
3	74.4	32.3	31.9	32.4	31.9	32.5 (32.1)	32.5 (31.3)	64.0	32.4
3-OCH_3	58.0								
4		126.3	126.8	126.4	126.9	126.4 (126.7)	126.3 (126.8)		126.3
5		132.4	132.4	132.4	132.4	132.0	132.5		132.5
6		72.7	72.9	72.7	72.9	72.7 (73.0)	73.5 (73.3)		63.2
6-OCH_3		59.1	59.0	59.1	59.0	59.2 (59.1)	59.2 (59.1)		
1′	129.7	132.0	132.5	132.8	133.3	132.3 (132.7)	135.4 (135.8)	130.0	135.7
2′,6′	128.8	129.9	129.5	129.8	129.4	128.4	129.1	128.7	129.1
3′,5′	116.4	116.1	116.1	116.0	116.0	116.2	116.2	116.3	116.0
4′	158.5	158.1	158.0	158.0	157.9	158.2	157.8	158.2	157.8
1″		130.9	131.0	130.9	131.0	130.9	130.8		130.9
2″,6″		128.1	128.1	128.1	128.1	129.9	128.1		128.2
3″,5″		116.2	116.3	116.2	116.3	116.1	115.9		116.2
4″		157.5	157.6	157.6	157.6	157.6	157.6		157.6
1‴						136.1			
2‴,6‴						128.2			
3‴,5‴						122.9			
4‴						151.6			
4‴-$OCOCH_3$						171.2			
4‴-$OCOCH_3$						21.0			
7‴						132.5			
8‴						127.9			
9‴						70.0 (70.1)			

[a] Measured in CD_3OD. [b] Letter **a** or **b** expressed stereoisomer. [c] Another stereoisomer in parentheses.

parameters were observed clearly. The ^1H NMR spectrum of each compound indicates the presence of four doublet proton signals (**2a**, δ 6.70, 6.80, 7.10, and 7.12; **2b**, δ 6.70, 6.78, 7.10, and 7.16), due to two para-substituted benzene rings; two *trans* olefinic proton signals [**2a**, δ 5.87 (td, $J = 7.3$, 15.7 Hz) and 6.14 (d, $J = 15.7$ Hz); **2b**, δ 5.99 (ddd, $J = 6.6$, 8.4, 15.7 Hz) and 6.28 (d, $J = 15.7$ Hz)]; two methylene protons (**2a**, δ 2.02, 2.09, 3.40, and 3.53; **2b**, δ 2.30, 2.46, 3.00, and 3.26); and two singlet methoxyl proton signals (**2a**, δ 3.14 and 3.31; **2b**, δ 3.16 and 3.18). The ^{13}C NMR spectrum showed 20 carbon signals including characteristic signals of two methoxyl carbons (**2a**, δ 56.8 and 59.1; **2b**, δ 57.0 and 59.0) and two phenolic carbons (**2a**, δ 157.5 and 158.1; **2b**, δ 157.6 and 158.0) (**Table 1**). Furthermore, the positions of two methoxyl groups were established on the basis of HMBC cross-peaks. A methoxyl proton signal (1-OCH_3: **2a**, δ 3.14; **2b**, δ 3.16) gave a strong cross-peak with a carbon signal (C-1: **2a**, δ 84.6; **2b**, δ 84.9), and another methoxyl proton signal (6-OCH_3: **2a**, δ 3.31; **2b**, δ 3.18) gave a strong cross-peak with a carbon signal (C-6: **2a**, δ 72.7; **2b**, δ 72.9). These results indicate that the two methoxyl groups of **2a** and **2b** are attached at the C-1 and C-6 positions. Thus, **2a** and **2b** were determined to be stereoisomers of (4*E*)-1,5-bis(4-hydroxyphenyl)-1-methoxy-2-(methoxymethyl)-4-pentene. The specific rotation (**2a**, -0.57; **2b**, $+1.0$) indicates each compound might be a mixture of enantiomers. Furthermore, compounds **2a** and **2b** are assumed to be diastereomers from the NMR data. However, the stereochemistry of the C-1 and C-2 positions of compounds **2a** and **2b** could not be established by NOESY experiments due to their flexible structures.

Compounds **3a** and **3b** were obtained as a yellow wax (11.7 mg) and a pale yellow wax (3.5 mg), respectively. The molecular formula of each compound was the same, determined to be $C_{21}H_{26}O_4$. The ^1H NMR spectra of compounds **3a** and **3b** were very similar to those of **2a** and **2b** except for the presence of signals due to an ethoxyl group (**3a**, δ 1.13, 3.25, and 3.34; **3b**, δ 1.15, 3.25, and 3.37). The ^{13}C NMR spectrum of compounds **3a** and **3b** showed 21 carbon signals including characteristic signals of a methoxyl carbon (**3a**, δ 59.1; **3b**, δ 59.0) and ethoxyl carbons (**3a**, δ 15.6 and 65.0; **3b**, δ 15.6 and 65.2) (**Table 1**). The positions of the methoxyl and ethoxyl groups were established by HMBC cross-peaks. A methyl proton signal (1-OCH_2CH_3: **3a**, δ 1.13; **3b**, δ 1.15) gave a strong cross-peak with a carbon signal (1-OCH_2CH_3: **3a**, δ 65.0; **3b**, δ 65.2), and this carbon signal then gave a cross-peak with a proton signal (H-1: **3a**, δ 4.21; **3b**, δ 4.22); a methylene proton signal (1-OCH_2CH_3: **3a**, δ 3.25 and 3.34; **3b**, δ 3.25 and 3.31) gave a cross-peak with a carbon signal (C-1: **3a**, δ 82.6; **3b**, δ 82.8); and a methoxyl proton signal (6-OCH_3: **3a**, δ 3.31; **3b**, δ 3.19) gave a strong cross-peak with a carbon signal (C-6: **3a**, δ 72.7; **3b**, δ 72.9). These results indicate that the ethoxyl group is attached at the C-1 position and the methoxyl group at C-6 position, respectively. Thus, the structures of compounds **3a** and **3b** were determined to be stereoisomers of (4*E*)-1,5-bis-(4-hydroxyphenyl)-1-ethoxy-2-(methoxymethyl)-4-pentene. Compounds **3a** and **3b** are assumed to be diastereomers from the NMR spectra. However, we could not determine the absolute configurations.

Compound **4** was obtained as a colorless wax (21.0 mg). The molecular formula was determined to be $C_{30}H_{32}O_6$. The ^1H NMR spectra of compound **3a** and **3b** were very similar to those of **2** and **3** except for the presence of signals due to an 4-(3-acetoxyphenyl)-2-propenoxyl group. The ^{13}C NMR spectrum showed 30 carbon signals including characteristic signals of acetyl carbons (δ 21.0 and 171.2), a methoxyl carbon (δ 59.1 or 59.2), and three phenolic carbons (δ 151.6, 157.6, and 158.2) (**Table 1**). The positions of the methoxyl and 3-(4-acetoxyphen-

yl)-2-propenoxyl groups in compound **4** were established by HMBC cross-peaks. A methoxyl proton signal at δ 3.31 or 3.18 (6-OCH_3) gave a strong cross-peak with a carbon signal at δ 72.7 or 73.0 (C-6), and methylene proton signals at δ 3.87 and 4.02 (H-9‴) gave strong cross-peaks with a carbon signal at δ 81.9 or 82.0 (C-1). These results indicate that the 3-(4-acetoxyphenyl)-2-propenoxyl group is attached at the C-1 position and the methoxyl group at C-6 position, respectively. From these spectroscopic data, compound **4** was determined to be (4*E*)-1,5-bis(4-hydroxyphenyl)-1-[(2*E*)-3-(4-acetoxyphenyl)-2-propenoxy]-2-(methoxymethyl)-4-pentene. The multiplicity of the NMR signals of **4** might be due to a mixture of diastereomers, but an attempt at further separation was not done.

Compound **5** was obtained as a colorless wax (14.0 mg). The molecular formula was determined to be $C_{19}H_{22}O_4$. The ^{1}H NMR spectrum was very similar to that of **2** except for the absence of one methoxyl group. The ^{13}C NMR spectrum showed 19 carbon signals including characteristic signals of one methoxyl carbon (δ 59.1 or 59.2) (**Table 1**). The position of the methoxyl group in compound **5** was established on the basis of HMBC cross-peaks. A methoxyl proton signal at δ 3.23 or 3.33 (6-OCH_3) gave a strong cross-peak with a carbon signal at δ 73.5 or 73.3 (C-6). This result indicates the methoxyl group is attached at the C-6 position. Thus, the structure of compound **5** was determined to be (4*E*)-1,5-bis(4-hydroxyphenyl)-2-(methoxymethyl)-4-penten-1-ol. The multiplicity of the NMR signals of **5** might be due to a mixture of diastereomers, although we did not separate each isomer.

Compound **6** was obtained as a white amorphous solid (350.0 mg). The structure of compound **6** was identified to be (*E*)-*p*-coumaryl alcohol [4-[(1*E*)-3-hydroxy-1-propenyl]phenol] by comparison of its ^{1}H and ^{13}C NMR data of **6** with those of the literature (*22*). (*E*)-*p*-Coumaryl alcohol has been found in the epicuticular wax of Gala apple fruits (*22*), and its acetyl derivative, (*E*)-*p*-coumaryl alcohol diacetate, has been isolated from the rhizomes of *A. galanga* as an inhibitor of xanthine oxidase (*23*).

Compound **7** was obtained as a colorless wax (16.0 mg). The molecular formula was determined to be $C_{18}H_{20}O_4$. The ^{1}H and ^{13}C NMR spectra of compound **7** were very similar to those of **2** except for the absence of two methoxyl groups (**Table 1**). The structural assignment of **7** was supported by HMBC cross-peaks (data not shown). From the spectroscopic data, the structure of compound **7** was determined to be (4*E*)-1,5-bis(4-hydroxyphenyl)-2-(hydroxymethyl)-4-penten-1-ol. The stereochemistry of **7** could not determined.

Antioxidative Activities of Compounds 1—7. Compounds **1**−**7** are expected to act as antioxidants because they have one or two phenolic hydroxyl groups in the molecule. **Figure 2** shows the inhibitory effect of compounds **1**−**7** during the autoxidation of methyl linoleate. Differences in antioxidative behavior of the isolated compounds were observed in their inhibition of methyl linoleate autoxidation. Although (*E*)-*p*-coumaryl γ-*O*-methyl ether (**1**) and (*E*)-*p*-coumaryl alcohol (**6**) each had one phenolic hydroxyl group, the antioxidative activity of **6** was higher than that of **1**. Compounds **2**−**5** exhibited lower antioxidative activity than that of α-tocopherol, a well-known chain-breaking antioxidant, whereas compound **7** had almost the same activity. These results indicate that the number of hydroxyl group in the phenylpropanoid molecule might influence its antioxidative activity. Further study will be carried out to obtain more information on their antioxidative mechanisms.

In this study, seven antioxidative compounds (**1**−**7**) were isolated from the rhizomes of smaller galanga (**Figure 1**). The most abundant antioxidants were (*E*)-*p*-coumaryl γ-*O*-methyl ether (**1**) and (*E*)-*p*-coumaryl alcohol (**6**). In addition, we have isolated five novel phenylpropanoids (**2**−**5** and **7**), which contain a novel $\beta-\gamma$ linkage in each molecule. Dilignols containing the $\beta-\gamma$ linkage structure have been previously reported (*24*, *25*). Although 1,2-dihydroxy-4-allylbenzene, a constituent of β-glycosides in smaller galanga (*16*), would be expected to be present as a candidate of antioxidant, we could not detect such a compound. Smaller galanga is used as a natural food ingredient in Vietnam and southern China. Therefore, consumption of its rhizome might be a good source of dietary antioxidants.

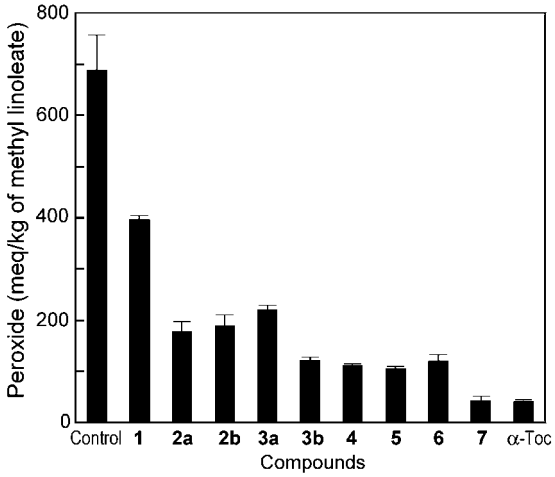

Figure 2. Inhibitory effect of compounds **1**–**7** on the autoxidation of methyl linoleate. Each value is expressed as the mean ± standard deviation of three different experiments. The letter **a** or **b** in **2a**, **2b**, **3a**, and **3b** expresses stereoisomers.

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