JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

DOI:10.1021/jf9010998

Coupling Reactions of Catechins with Natural Aldehydes and Allyl Alcohols and Radical Scavenging Activities of the Triglyceride-Soluble Products

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Hydrophobic catechin derivatives were produced by heating with natural aldehydes or allyl alcohols. (+)-Catechin or (−)-epigallocatechin-3-*O*-gallate was heated with *trans*-2-hexenal, citral, (+)-citronellal, geraniol, or phytol. Although each reaction generated complex mixtures of products, 11 compounds were isolated and characterized by spectroscopic methods. The unsaturated aldehydes were found to attach to the flavan A-ring. Besides C−C linkage between aldehyde and the C-8 and/or C-6 of the catechin A-ring, formation of ether linkages between unsaturated carbons of the aldehydes and phenolic hydroxyl groups was observed. The allyl alcohols, geraniol and phytols, reacted at the galloyl group as well as the A-ring. After partitioning between triglyceride and water, the lipid layer of the reaction products showed strong 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. In contrast, epigallocatechin-3-*O*-gallate was not transferred to the lipid layer.

KEYWORDS: Catechin; essential oil; phytol; aldehyde; allyl alcohol; radical scavenging

INTRODUCTION

Recent medical and biological studies indicate that oxidative stress causes and exacerbates some chronic diseases, such as atherosclerosis, diabetes, and cancer (*1*−*3*). In addition, epidemiological studies strongly suggest a correlation between a higher content of polyphenols in the diet and a lower risk of mortality from cancer and coronary heart diseases (*4*−*7*). Therefore, polyphenols as antioxidative food constituents have attracted the attention of food scientists, manufacturers, and consumers. Catechins, such as (+)-catechins (**1**) and (−)-epigallocatechin-3-*O*-gallate (**2**), are known to prevent free radical-mediated damage (*8*−*11*). However, the bioavailability of tea catechins and related tea polyphenols is not sufficiently high to display their antioxidative abilities observed in in vitro experiments (*12*−*14*). Tea catechins are water-soluble antioxidants. In addition, tea catechins associate with proteins and lipid bilayers through hydrophobic interactions and hydrogen bonding (*15*−*18*). To alter the solubility and physicochemical nature by chemical methods, some derivatives of tea catechins have been synthesized (*19*−*22*). Although direct application of these compounds as food additives may be difficult because of the technical difficulties associated with production and the current lack of safety evaluations available, these derivatives are interesting from the viewpoint of medicinal and food chemistry. The aim of this study is to develop a new method to prepare catechin derivatives without using special chemical reagents that are unacceptable as food ingredients; thus, this paper introduces a facile method to produce catechin derivatives with hydrophobic moieties. Some of the products showed increased lipid solubility and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities in the triglyceride (TG) layer following TG/water partitioning of the products.

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MATERIALS AND METHODS

Materials. (+)-Catechin (**1**) was isolated from commercial gambir (extract of the leaves and young twigs of *Uncaria gambir*) and purified by recrystallization (*23*). (−)-Epigallocatechin-3-*O*-gallate (**2**) was isolated from commercial green tea by column chromatography and purified by crystallization (*24*). Essential oils and phytol were purchased from Tokyo Chemical Industry Co., Ltd., Japan, and other chemicals and solvents were of reagent grade.

Analytical Procedures. UV spectra were obtained using a JASCO V-560 UV−vis spectrophotometer. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. The ^{1}H and ^{13}C NMR, ^{1}H−^{1}H correlation spectroscopy (COSY), NOE spectroscopy (NOESY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple-bond connectivity (HMBC) spectra were recorded in acetone-d_6 at 27 °C on a Unity *plus* 500 spectrometer (Varian, Palo Alto, CA) operating at a ^{1}H frequency of 500 MHz. The one-dimensional ^{1}H and ^{13}C NMR spectra were recorded with a JEOL JNM-AL400 spectrometer operating at a ^{1}H frequency of 400 MHz. The matrix-assisted laser desorption time-of-flight mass spectra (MALDI TOF MS) were recorded on a Voyager-DE Pro spectrometer (Applied Biosystems, Fullerton, CA), and 2,5-dihydrohydroxybenzoic acid (10 mg/mL in 50% acetone containing 0.05% trifluoroacetic acid) was used as the matrix. Fast atom bombardment (FAB) MS was recorded on a JMS 700N spectrometer (JEOL Ltd., Japan), and

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Published on Web 06/19/2009

m-nitrobenzyl alcohol or glycerol was used as the matrix. Elemental analysis was conducted with a Perkin-Elmer 2400α analyzer (Perkin-Elmer Inc., Waltham, MA).

Column chromatography was performed using Sephadex LH-20 (25−100 μm, GE Healthcare Bio-Science AB, Uppsala, Sweden), Chromatorex ODS (100−200 mesh; Fuji Silysia Chemical Ltd., Tokyo, Japan), and silica gel 60N (100−210 μm, Kanto Chemical Co., Inc., Tokyo, Japan) columns. Thin layer chromatography (TLC) was performed on precoated Kieselgel 60 F_{254} plates (0.2 mm thick, Merck, Darmstadt, Germany) with toluene/ethyl formate/formic acid (5:4:1 or 1:7:1 v/v) or $CHCl_3$/MeOH/water (90:10:1, 80:20:2, or 70:30:5 v/v). Spots were detected using ultraviolet (UV) illumination and by spraying the plates with 2% ethanolic $FeCl_3$ or 10% sulfuric acid reagents followed by heating. Analytical HPLC was performed on a Cosmosil $5C_{18}$-AR II (Nacalai Tesque Inc., Tokyo, Japan) column (4.6 mm i.d. × 250 mm) with a gradient elution from 25 to 90% (0−40 min) and 90% (40−55 min) of CH_3CN in 50 mM H_3PO_4. A flow rate of 0.8 mL/min was used. The column temperature was maintained at 35 °C, and detection was achieved using a JASCO photodiode array detector MD-910. Preparative HPLC was performed on a Cosmosil $5C_{18}$-AR-II column (20 × 250 mm) with 90% CH_3CN containing 0.1% TFA.

General Reaction Procedures. Aldehyde or allyl alcohol (4.0 g) was added to a solution of **1** or **2** in acetone (2.0 g/30 mL), and the solvent was evaporated under reduced pressure using a rotary evaporator. In the case of the reaction with geraniol and phytol, citric acid (0.4 g) and 0.1 M ethanolic HCl (0.4 mL), respectively, were added prior to evaporation. The resulting syrup was left standing at room temperature or heated (50 or 80 °C), and the reaction was monitored by TLC analysis. The mixture was first separated by Sephadex LH-20 column chromatography with EtOH. The fractions were monitored by TLC and separated into several fractions containing starting materials and products. The fractions containing the products were subjected to Chromatorex ODS column chromatography with 40−100% MeOH (10% stepwise gradient elution). Generally, the reactions afforded a complex mixture of the products including diastereomeric isomers and, thus, the yields of the purified compounds were significantly low. Reaction of **1** (2.1 g) with *trans*-2-hexanal (4.0 g) at room temperature for 1 week yielded **3** (113 mg). Reaction of **2** (2.0 g) with citral (4.1 g) at 100 °C for 20 h yielded **4** (52.2 mg), **5** (70.5 mg), **6** (68.4 mg), and **7** (38.6 mg). Reaction of **2** (2.0 g) with (+)-citronellal (4.0 g) at 80 °C for 5 h yielded **8** (377 mg) and **9** (56.4 mg). Reaction of **2** (2.0 g) with geraniol (4.0 g) in the presence of citric acid (0.4 g) at 100 °C for 5 h yielded **11** (39.3 mg) and **12** (16.5 mg). Reaction of **2** (4.0 g) with phytol (2.0 g) in the presence of 0.1 M HCl (0.4 mL) at 50 °C for 30 h yielded **13** (48.0 mg) and **14** (70.0 mg). Reaction of phloroglucinol (2.0 g) with (+)-citronellal (0.4 g) at 80 °C for 5 h yielded **10** (210.0 mg). Purification of product **10** was performed by silica gel column chromatography with $CHCl_3$/MeOH/H_2O (95:5:0 and 90:10:1).

Compound **3** was obtained as a brown amorphous powder: $[\alpha]_D^{29}$ −59.4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} nm (ε) 280 (7280); IR (dry film) ν_{max} cm^{-1} 3389, 1692, 1616, 1449; ^{1}H NMR (500 MHz, acetone-d_6) δ 0.42 (3H, t, *J* = 7.3, Ha-6″), 0.69 (3H, t, *J* = 7.3, Hb-6″), 0.98 (1H, m, Ha-5″), 1.06 (1H, m, Ha-5″), 1.16 (1H, m, Hb-5″), 1.18 (1H, m, Ha-4″), 1.29 (1H, m, Hb-5″), 1.31 (1H, m, Hb-4″), 1.55 (1H, m, Ha-4″), 1.72 (1H, m, Hb-4″), 1.93 (2H, m, H-2″), 2.56 (2H, m, Ha-4, Hb-4), 2.78 (1H, m, Hb-3″), 2.80 (1H, m, Ha-3″), 2.94 (2H, m, Ha-4′, Hb-4′), 3.92 (2H, m, Ha-3, Hb-3), 4.00 (2H, m, Ha-3′, Hb-3′), 4.54, 4.54 (each 1H, d, *J* = 8.2 Hz, Ha, b-2), 4.59, 4.63 (each 1H, d, *J* = 7.8 Hz, Ha, b-2′), 5.50 (1H, dd, *J* = 2.5, 11.7 Hz, H-1″), 5.53 (1H, dd, *J* = 2.5, 11.7 Hz, H-1″), 6.03 (2H, s, H-6, H-6′), 6.76 (4H, m, B-ring H-5,5′,6,6′), 6.90 (2H, m, B-ring H-2,2′), 7.64 (1H, s, OH-7), 7.72 (1H, s, OH-7), 7.89 (4H, m, B-ring OH-3,3′4,4′), 8.33 (1H, s, OH-5′), 8.34 (1H, s, OH-5′), 8.40 (1H, s, OH-5), 8.40 (1H, s, OH-5); ^{13}C NMR (125 MHz, acetone-d_6) δ 14.0 (Ca-6″), 14.2 (Cb-6″), 20.9 (Ca-5″), 21.2 (Cb-5″), 28.8 (Ca-4, Cb-4), 28.9 (Ca-4′, Cb-4′), 30.0 (Cb-3″), 30.2 (Ca-3″), 31.1 (Ca-2″), 31.5 (Cb-2″), 37.6 (Ca-4″), 38.0 (Cb-4″), 67.84, 67.9 (Ca-3′, Cb-3′), 68.3, 68.6 (Ca-3, Cb-3), 70.6, 71.5 (Ca-1″, Cb-1″), 82.3, 83.0 (C-2, C-2′), 95.9, 96.1, 96.9, 97.0 (Ca-6, Cb-6, Ca-6′, Cb-6′), 101.0, 102.3, 102.7 (Ca-4, Ca-4′, Cb-4, Cb-4′), 105.1, 105.5 (C-8, C-8′), 107.4, 107.7 (C-8a, C-8a′), 114.9, 115.20, 115.2 (B-ring C-2,2′), 115.2, 115.5, 115.6, 115.6 (B-ring C-5,5′), 119.6, 119.7, 119.9, 119.9 (B-ring C-6,6′), 131.6, 131.8, 132.0, 132.1 (B-ring C-1,1′), 145.7, 145.5, 145.5, 145.6, 145.7 (B-ring C-3,3′,4,4′), 153.1, 153.2 (Ca-8a′, Cb-8a′), 153.3 (C-8a), 153.9, 154.0 (Ca-5′, Cb-5′), 155.9, 156.0 (C-7, C-7′), 156.4 (C-5); MALDI TOF MS *m*/*z* 683 [M + Na]$^+$, 699 [M + K]$^+$. Anal. Calcd for $C_{36}H_{36}O_{12}\cdot {}^{9}/_{4}\,H_2O$: C, 61.67%; H, 5.82%. Found: C, 61.73%; H, 5.76%.

Compound **4** was obtained as a brown amorphous powder: $[\alpha]_D^{29}$ −248.3 (*c* 0.1 MeOH); UV (MeOH) λ_{max} nm (ε) 276 (10050); IR (dry film) ν_{max} cm^{-1} 3390, 1694, 1615, 1537, 1448; ^{1}H NMR (500 MHz, acetone-d_6) δ 0.85 (3H, s, H-9′), 0.95 (3H, s, H-8′), 1.29 (1H, m, H-5b′), 1.29 (3H, s, H-10′), 1.52 (1H, br d, *J* = 14.9 Hz, H-5a′), 1.58 (1H, dd, *J* = 4.8, 12.3 Hz, H-4b′), 1.65 (1H, br d, *J* = 12.6 Hz, H-6′), 1.73 (1H, dt, *J* = 3.0, 12.8 Hz, H-2b′), 1.90 (2H, br d, *J* = 12.8 Hz, H-2a′, H-4a′), 2.95 (1H, dd, *J* = 2.2, 17.3 Hz, H-4b), 3.09 (1H, br d, *J* = 17.3 Hz, H-4a), 3.43 (1H, br s, OH-7′), 3.64 (1H, br d, *J* = 2.3 Hz, H-1′), 5.15 (1H, br s, H-2), 5.49 (1H, br s, H-3), 6.01 (1H, s, H-6), 6.67 (2H, s, B-ring H-2,6), 7.02 (2H, s, galloyl H-2,6), 8.36 (1H, br s, OH-5); ^{13}C NMR (125 MHz, acetone-d_6) δ 22.3 (C-5′), 25.7 (C-9′), 26.5 (C-4), 27.4 (C-1′), 29.0 (C-8′,10′), 39.4 (C-2′), 40.9 (C-4′), 54.7 (C-6′), 69.4 (C-3), 72.1 (C-7′), 75.0 (C-3′), 78.5 (C-2), 96.3 (C-6), 98.7 (C-4a), 104.2 (C-8), 106.7 (B-ring C-2,6), 110.0 (galloyl C-2,6), 121.7 (galloyl C-1), 129.9 (B-ring C-1), 133.3 (B-ring C-4), 138.7 (galloyl C-4), 145.8 (galloyl C-3,5), 146.3 (B-ring C-3,5), 152.3 (C-8a), 155.6 (C-5), 156.9 (C-7), 166.0 (galloyl C-7); MALDI TOF MS *m*/*z* 633 [M + Na]$^+$, 649 [M + K]$^+$. Anal. Calcd for $C_{32}H_{34}O_{12}\cdot 2H_2O$: C, 59.49%; H, 5.92%. Found: C, 59.58%; H, 6.12%.

Compound **5** was obtained as a brown amorphous powder: $[\alpha]_D^{29}$ −135.7 (*c* 0.1 MeOH); UV (MeOH) λ_{max} nm (ε) 276 (9450); IR (dry film) ν_{max} cm^{-1} 3405, 1693, 1618, 1537, 1449; ^{1}H NMR (500 MHz, acetone-d_6) δ 1.05 (3H, s, H-8′), 1.21 (3H, s, H-9′), 1.27 (3H, s, H-10′), 1.32 (1H, dd, *J* = 4.2, 12.9 Hz, H-5′), 1.53 (2H, m, H-4′, H-5′), 1.60 (1H, br d, *J* = 12.8 Hz, H-5′), 1.71 (1H, br d, *J* = 12.8 Hz, H-6′), 1.86 (1H, dd, *J* = 3.0, 15.1 Hz, H-2′), 1.91 (1H, m, H-4′), 2.95 (1H, br d, *J* = 18.0 Hz, H-4), 3.10 (1H, dd, *J* = 5.3, 18.0 Hz, H-4), 3.51 (1H, br s, H-1′), 4.99 (1H, br s, H-2), 5.51 (1H, d, *J* = 5.3 Hz, H-3), 6.05 (1H, s, H-6), 6.71 (2H, s, B-ring H-2,6), 7.15 (2H, s, galloyl H-2,6); ^{13}C NMR (125 MHz, acetone-d_6) δ 22.5 (C-5′), 25.9 (C-8′), 27.3 (C-1′), 27.7 (C-4), 28.9 (C-10′), 29.9 (C-9′), 39.3 (C-2′), 40.8 (C-4′), 54.1 (C-6′), 69.2 (C-3), 73.4 (C-7′), 75.0 (C-3′), 80.4 (C-2), 96.6 (C-6), 99.5 (C-4a), 104.7 (C-8), 107.6 (B-ring C-2,6), 110.2 (galloyl C-2,6), 121.6 (galloyl C-1), 129.3 (B-ring C-1), 133.8 (B-ring C-4), 138.9 (galloyl C-4), 145.8 (galloyl C-3,5), 146.3 (B-ring C-3,5), 153.0 (C-8a), 155.7 (C-5), 156.9 (C-7), 166.4 (galloyl C-7); MALDI TOF MS *m*/*z* 633 [M + Na]$^+$, 649 [M + K]$^+$. Anal. Calcd for $C_{32}H_{34}O_{12}\cdot 2H_2O$: C, 58.62%; H, 6.00%. Found: C, 58.63%; H, 6.19%.

Compound **6** was obtained as a brown amorphous powder: $[\alpha]_D^{29}$ −79.2 (*c* 0.1 MeOH); UV (MeOH) λ_{max} nm (ε) 276 (9880); IR (dry film) ν_{max} cm^{-1} 3409, 1692, 1618, 1536, 1448; ^{1}H NMR (500 MHz, acetone-d_6) δ 0.47 (1H, m, H-5′), 1.00 (3H, s, H-8′), 1.11 (1H, m, H-4′), 1.28 (3H, s, H-10′), 1.39 (2H, m, H-4′, H-5′), 1.44 (3H, s, H-9′), 1.87 (1H, dd, *J* = 1.6, 13.0 Hz, H-2′), 2.00 (1H, m, H-6′), 2.09 (1H, m, H-2′), 2.77 (1H, br s, H-1′), 2.98 (2H, m, H-4), 5.03 (1H, br s, H-2), 5.51 (1H, dd, *J* = 1.4, 3.4 Hz, H-3), 6.07 (1H, s, H-8), 6.63 (2H, s, B-ring H-3,5), 6.98 (2H, s, galloyl H-2,6); ^{13}C NMR (125 MHz, acetone-d_6) δ 22.6 (C-5′), 24.0 (C-9′), 26.2 (C-4), 28.9 (C-1′), 29.2 (C-10′), 29.9 (C-8′), 35.7 (C-2′), 38.0 (C-4′), 47.2 (C-6), 69.1 (C-3), 74.5 (C-3′), 78.2 (C-2), 84.1 (C-7′), 98.7 (C-8), 101.1 (C-4a), 106.6 (B-ring C-2,6), 109.8 (galloyl C-2,6), 110.00 (C-6), 121.9 (galloyl C-1), 130.7 (B-ring C-1), 133.0 (B-ring C-4), 138.6 (galloyl C-4), 145.8 (galloyl C-3,5), 146.2 (B-ring C-3,5), 155.1 (C-8a), 155.8 (C-5), 156.0 (C-7), 166.1 (galloyl C-7); MALDI TOF MS *m*/*z* 633 [M + Na]$^+$, 649 [M + K]$^+$. Anal. Calcd for $C_{32}H_{34}O_{12}\cdot {}^{3}/_{2}H_2O$: C, 60.28%; H, 5.85%. Found: C, 60.20%; H, 5.94%.

Compound **7** was obtained as a brown amorphous powder: $[\alpha]_D^{29}$ 52.2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} nm (ε) 281 (13170); IR (dry film) ν_{max} cm^{-1} 3389, 1692, 1614, 1536, 1446; ^{1}H NMR (500 MHz, acetone-d_6) δ 1.30 (3H, s, H-10′), 1.57 (3H, d, *J* = 0.7 Hz, H-8′), 1.63 (3H, d, *J* = 0.7 Hz, H-9′), 1.67 (2H, t, *J* = 8.2 Hz, H-4′), 2.10 (2H, m, H-5′), 2.87 (1H, dd, *J* = 2.2, 17.4 Hz, H-4), 3.02 (1H, dd, *J* = 4.8, 17.4 Hz, H-4), 5.05 (1H, s, H-2), 5.11 (1H, m, H-6′), 5.41 (1H, d, *J* = 10.0 Hz, H-2′), 5.54 (1H, m, H-3), 6.07 (1H, s, H-8), 6.59 (1H, s, B-ring H-2,6), 6.63 (1H, d, *J* = 10.0 Hz, H-1′), 7.01 (2H, s, galloyl H-2,6); ^{13}C NMR (125 MHz, acetone-d_6) δ 17.6 (C-8′), 23.4 (C-5′), 25.8 (C-9′), 26.3 (C-4), 26.5 (C-10′), 41.7 (C-4′), 69.0 (C-3), 78.2 (C-2), 79.1 (C-3′), 96.0 (C-8), 99.8 (C-4a), 103.9 (C-6), 106.6 (B-ring C-2,6), 109.9 (galloyl C-2,6), 118.3 (C-1′), 121.7 (galloyl C-5), 124.9 (C-2′), 125.1 (C-6′), 130.4 (B-ring C-1), 131.8 (C-7′), 133.1 (B-ring C-4), 138.7 (galloyl C-4), 145.8 (galloyl C-3,5),

146.2 (B-ring C-3,5), 152.8 (C-7), 153.2 (C-5), 156.4 (C-8a), 166.0 (gallyol C-7); MALDI TOF MS m/z 615 $[M + Na]^+$. Anal. Calcd for $C_{32}H_{32}O_{11}\cdot{}^5/_2H_2O$: C, 60.28%; H, 5.85%. Found: C, 60.25%; H, 5.96%.

*Compound **8*** was obtained as a tan amorphous powder: $[\alpha]_D^{22}$ −197.7 (c 0.1, MeOH); UV (MeOH) λ_{max} nm (ε) 275 (12393); IR (dry film) ν_{max} cm^{-1} 3401, 2921, 2865, 1693, 1608, 1536, 1453; ^{1}H NMR (acetone-d_6, 500 MHz) δ 0.64 (m, H-2a′), 1.30, 1.38 (s, H-8′), 5.06, 5.08 (br s, H-2), 5.55 (m, H-3), 6.66 (d, J = 0.5 Hz, H-B-2,6), 6.96 (2H, s, galloyl H-2,6); ^{13}C NMR (acetone-d_6, 125 MHz) δ 19.3 (C-9), 22.9, 23.1 (C-10′), 26.9, 27.0 (C-4), 27.9, 28.0 (C-8′), 28.7, 28.8 (C-5′), 33.6, 33.7 (C-3′), 36.4, 36.5 (C-1′, C-4′), 40.5, 40.8 (C-2′), 50.4 (C-6′), 69.4 (C-3), 77.1, 77.2, 77.3 (C-2, C-7′), 100.3 (C-4), 106.8, 106.9, 107.5 (C-6, C-8), 106.1 (B-ring C-2,6), 109.9, 110.1 (galloyl C-2,6), 122.0 (galloyl C-1), 130.9 (B-ring C-1), 132.7, 132.8 (B-ring C-4), 138.6, 138.8 (galloyl C-4), 145.8, 146.3 (galloyl C-3, 5, B-ring C-3, 5), 151.2, 152.8, 153.0 (C-5, C-7, C-8a), 166.2 (galloyl C-7); MALDI TOF MS m/z 753 $[M + Na]^+$. Anal. Calcd for $C_{42}H_{50}O_{11}\cdot{}^3/_2H_2O$: C, 66.56%; H, 7.05%. Found: C, 66.59%; H, 7.03%.

*Compound **9*** was obtained as a brown amorphous powder: $[\alpha]_D^{20}$ −200.6 (c 0.1, MeOH); UV (MeOH) λ_{max} nm (ε) 276 (11597); IR (dry film) ν_{max} cm^{-1} 3373, 1682, 1615, 1455; ^{1}H NMR (acetone-d_6, 500 MHz) δ 0.62, 0.64 (q, J = 11.5 Hz, H-2a′), 0.81, 0.86 (d, J=9.8 Hz, H-10′), 1.04, 1.07 (s, H-9′), 1.06 (m, H-4a′), 1.13 (m, H-5a′), 1.30, 1.31 (s, H-8′), 1.37, 1.39 (m, H-6′), 1.58 (m, H-3′), 1.82 (m, H-4b′, H-5b′), 2.48, 2.55 (dt, J=2.5, 11.0 Hz, H-1′), 2.93 (dd, J = 2.4, 14.0 Hz, H-4a), 3.02, 3.19 (br d, J = 13.1 Hz, H-2b′), 3.08 (dd, J = 4.5, 14.0 Hz, H-4b), 5.03, 5.15 (br s, H-2), 5.54, 5.56 (m, H-3), 5.94, 5.95 (s, H-6), 6.61, 6.66 (d, J = 0.5 Hz, H-B-2,6), 6.99, 7.02 (2H, s, galloyl-2,6); ^{13}C NMR (acetone-d_6, 125 MHz) δ 19.1, 19.3 (C-9), 22.8, 23.0 (C-10′), 26.6, 26.9 (C-4), 28.7, 28.8 (C-8′), 28.7, 28.8 (C-5′), 33.3, 33.6 (C-3′), 36.1, 36.3 (C-1′), 36.2, 36.3 (C-4′), 39.9, 40.3 (C-2′), 50.4 (C-6′), 69.0, 69.3 (C-3), 77.1, 77.7 (C-2), 77.1, 77.2 (C-7′), 97.4, 97.7 (C-6), 100.0, 100.4 (C-4), 105.9, 107.6 (C-8), 106.1, 106.6 (B-ring C-2,6), 109.8, 109.9 (galloyl C-2,6), 121.6, 121.8 (galloyl C-1), 130.5, 130.6 (B-ring C-1), 132.8, 133.0 (B-ring C-4), 138.6, 138.7 (galloyl C-4), 145.8 (galloyl C-3,5), 146.1, 146.2 (B-ring C-3,5), 154.1, 154.2 (C-7), 154.7, 154.9 (C-8a), 155.2 (C-5), 166.0, 166.1 (galloyl C-7); MALDI TOF MS m/z 617 $[M + Na]^+$, 633 $[M + K]^+$. Anal. Calcd for $C_{32}H_{34}O_{11}\cdot{}^3/_2H_2O$: C, 61.83%; H, 6.00%. Found: C, 61.82%; H, 6.41%.

*Compound **10*** was obtained as a white amorphous powder: ^{1}H NMR (500 MHz, acetone-d_6) δ 0.62 (1H, q, J = 11.5 Hz, H-2′), 0.90 (3H, d, J= 6.6 Hz, H-10′), 1.01 (3H, s, H-9′), 1.08 (1H, m, H-4′), 1.11 (1H, m, H-5′), 1.28 (3H, s, H-8′), 1.34 (1H, m, H-6′), 1.57 (1H, m, H-3′), 1.83 (2H, m, H-4′, H-5′), 2.37 (1H, dt, J=2.8, 11.5 Hz, H-1′), 3.19 (1H, br d, J=11.5 Hz, H-2′), 5.76 (1H, d, J=2.5 Hz, H-5), 5.92 (1H, d, J=2.5 Hz, H-3), 7.94 (1H, s, C-4-OH), 8.17 (1H, s, C-2-OH); ^{13}C NMR (500 MHz, acetone-d_6) δ 19.2 (C-9′), 23.0 (C-10′), 28.0 (C-8′), 28.7 (C-5′), 33.4 (C-3′), 36.2 (C-1′), 36.3 (C-4′), 40.0 (C-2′), 50.3 (C-6′), 77.0 (C-7′), 96.4 (C-5), 96.6 (C-3), 105.2 (C-1), 156.4 (C-6), 157.4 (C-4), 158.1(C-2); MALDI TOF MS m/z 263 $[M+H]^+$, 285 $[M+Na]^+$.

*Compound **11*** was obtained as a brown amorphous powder: $[\alpha]_D^{20}$ − 123.6 (c 0.1, MeOH); UV (MeOH) λ_{max}nm (ε) 276 (11286); IR (dry film) ν_{max} cm^{-1} 3389, 1691, 1619, 1454; ^{1}H NMR (500 MHz, acetone-d_6) δ 1.53 (3H, d, J=0.9 Hz, H-10′), 1.59 (3H, d, J=1.1 Hz, H-8′), 1.70 (3H, d, J = 1.1 Hz, H-9′), 1.94 (2H, m, H-4′), 2.00 (2H, m, H-5′), 2.90 (1H, dd, J=2.1, 15.0 Hz, H-4a), 3.02 (1H, dd, J=4.8, 15.0 Hz, H-4b), 3.32 (1H, dd, J=6.5, 14.0 Hz, H-1a′), 3.42 (1H, dd, J=7.6, 14.0 Hz, H-1b′), 5.07 (2H, m, H-2 and H-6′), 5.34 (1H, br t, J = 7 Hz, H-2′), 5.54 (1H, br s, H-3), 6.10 (1H, s, H-6), 6.66 (2H, s, B-ring H-2,6), 7.01 (2H, s, galloyl H-2,6); ^{13}C NMR (125 MHz, acetone-d_6) δ 16.3 (C-9′), 17.7 (C-10′), 22.5 (C-1′), 25.8 (C-8′), 26.7 (C-4), 27.5 (C-5′), 40.5 (C-4′), 69.2 (C-3), 77.9 (C-2), 96.2 (C-6), 98.9 (C-4a), 106.5 (B-ring C-2,6), 107.8 (C-8), 110.0 (galloyl C-6), 121.8 (galloyl C-1), 124.9 (C-2′), 125.3 (C-6′), 130.9 (B-ring C-1), 131.3 (C-7′), 132.9 (B-ring C-4), 134.1 (C-3′), 138.7 (galloyl C-4), 145.8 (galloyl C-3,5), 146.2 (B-ring C-3,5), 154.4 (C-8a), 154.5 (C-5), 154.8 (C-7), 166.1 (galloyl C-7); MALDI TOF MS m/z 617 $[M + Na]^+$. Anal. Calcd for $C_{32}H_{34}O_{11}\cdot 2H_2O$: C, 60.95%; H, 6.07%. Found: C, 61.03%; H, 6.10%.

*Compound **12*** was obtained as a brown amorphous powder: $[\alpha]_D^{20}$ −92.6 (c 0.1, MeOH); UV (MeOH) λ_{max}nm (ε) 273 (9801); IR (dry film) ν_{max} cm^{-1} 3399, 1686, 1607, 1465; ^{1}H NMR (500 MHz, acetone-d_6) δ 1.52 (3H, d, J=0.9 Hz, H-10′), 1.58 (3H, d, J=1.2 Hz, H-8′), 1.68 (3H, d, J=1.2 Hz, H-9′), 1.86 (2H, t, J = 7.2 Hz, H-4′), 1.99 (2H, m, H-5′), 2.89 (1H, dd, J = 2.5, 17.6 Hz, H-4), 3.03 (1H, dd, J = 4.8, 17.6 Hz, H-4), 3.56 (1H, dd, J=6.0, 14.0 Hz, H-1a′), 3.64 (1H, dd, J=7.6, 14.0 Hz, H-1b′), 5.05 (2H, br s, H-2 and H-6′), 5.12 (1H, br t, J = 6.5 Hz, H-2′), 5.55 (1H, br s, H-3), 5.98 (1H, d, J=2.3 Hz, H-8), 6.04 (1H, d, J=2.3 Hz, H-6), 6.60 (2H, s, B-ring H-2,6), 6.93 (1H, s, galloyl H-6); ^{13}C NMR (125 MHz, acetone-d_6) δ 16.3 (C-9′), 17.7 (C-10′), 23.6 (C-1′), 25.5 (C-8′), 26.5 (C-4), 27.4 (C-5′), 40.5 (C-4′), 69.0 (C-3), 77.9 (C-2), 95.7 (C-8), 96.3 (C-6), 99.1 (C-4a), 106.7 (B-ring C-2,6), 110.4 (galloyl C-6), 121.1 (galloyl C-1), 124.1 (galloyl C-2), 124.9 (C-2′), 125.3 (C-6′), 130.6 (B-ring C-1), 131.8 (C-7′), 133.1 (B-ring C-4), 134.3 (C-3′), 137.4 (galloyl C-4), 142.8 (galloyl C-5), 144.8 (galloyl C-3), 146.1 (B-ring C-3,5), 156.9 (C-8a), 157.4 (C-5), 157.6 (C-7), 167.0 (galloyl C-7); MALDI TOF MS m/z 617 $[M + Na]^+$. Anal. Calcd for $C_{32}H_{34}O_{11}\cdot 2H_2O$: C, 60.95%; H, 6.07%. Found: C, 60.95%; H, 6.09%.

*Compound **13*** was obtained as a brown amorphous powder: $[\alpha]_D^{20}$ −79.2 (c 0.1, MeOH); UV (MeOH) λ_{max} nm (ε) 276 (12358); IR (dry film) ν_{max} cm^{-1} 3425, 1694, 1626, 1455; ^{1}H NMR (500 MHz, acetone-d_6) δ 0.83, 0.84 (each 3H, d, J = 6.6 Hz, H-18′, H-19′), 0.86 (6H, d, J = 6.6 Hz, H-16′, H-20′), 1.02−1.41 (m, H-5′−H-14′), 1.52 (1H, septet, J = 6.6 Hz, H-15′), 1.74 (3H, d, J = 0.9, H-17′), 1.91 (2H, m, H-4′), 2.91 (1H, dd, J = 2.1, 17.2 Hz, C-4a), 3.08 (1H, dd, J = 4.8, 17.2 Hz, H-4b), 3.33 (2H, m, H-1′), 5.01 (1H, s, H-2), 5.24 (1H, br t, J = 7.1 Hz, H-2′), 5.54 (1H, m, H-3), 6.13 (1H, s, H-8), 6.60 (2H, d, J = 0.5 Hz, B-ring H-2,6), 7.00 (1H, s, galloyl H-2,6), 7.11 (1H, s, 5-OH), 8.16 (1H, s, 7-OH); ^{13}C NMR (125 MHz, acetone-d_6) δ 16.1 (C-17′), 20.0 (C-16′,20′), 22.8, 22.9 (C-18′,19′), 22.6 (C-1′), 27.0 (C-4), 25.1, 25.4, 26.0, 28.6, 33.3, 33.4, 37.4, 37.9, 38.0, 38.1, 40.0 (C-5′−C-15′), 40.7 (C-4′), 69.2 (C-3),77.7 (C-2), 96.2 (C-8), 99.4 (C-4a), 106.6 (B-ring C-2.6), 108.9 (C-6), 109.9 (galloyl C-2,6), 121.7 (galloyl C-1), 124.2 (C-2′), 130.6 (B-ring C-1), 133.1 (B-ring C-4), 135.1 (C-3′), 138.7 (galloyl C-4), 145.8 (galloyl C-3,5), 146.2 (B-ring C-3,5), 154.2 (C-8a), 154.7 (C-5), 155.0 (C-7), 167.0 (galloyl C-7); MALDI TOF MS m/z 759 $[M + Na]^+$. Anal. Calcd for $C_{42}H_{56}O_{11}\cdot{}^1/_4H_2O$: C, 68.04%; H, 7.68%. Found: C, 68.05%; H, 8.06%.

*Compound **14*** was obtained as a brown amorphous powder: $[\alpha]_D^{19}$ −84.9 (c 0.1, MeOH); UV (MeOH) λ_{max} nm (ε) 272 (10106); IR (dry film) ν_{max} cm^{-1} 3379, 1692, 1608, 1462; ^{1}H NMR (500 MHz, acetone-d_6) δ 0.82, 0.86 (each 3H, d, J = 6.6 Hz, H-18′, H-19′), 0.85 (6H, d, J = 6.6 Hz, H-19′, H-20′), 1.02−1.41 (m, H-5′−H-17′), 1.52 (1H, septet, J = 6.6 Hz, H-15′), 1.67 (3H, d, J = 0.9 Hz, H-17′), 1.85 (2H, m, H-4′), 2.89 (1H, dd, J = 2.3, 17.4 Hz, C-4a), 3.02 (1H, dd, J = 4.5, 17.4 Hz, H-4b), 3.56 (1H, dd, J = 6.6, 14.0 Hz, H-1a′), 3.64 (1H, dd, J = 6.6, 14.0 Hz, H-1b′), 5.05 (1H, s, H-2), 5.12 (1H, br t, J = 6.9 Hz, H-2′), 5.55 (1H, m, H-3), 5.98 (1H, d, J = 2.3 Hz, H-8), 6.04 (1H, d, J = 2.3 Hz, H-6), 6.60 (2H, d, J = 0.7 Hz, B-ring H-6), 6.92 (1H, s, galloyl H-6), 8.13 (1H, br s, 7-OH), 8.32 (1H, br s, 5-OH); ^{13}C NMR (125 MHz, acetone-d_6) δ 16.2 (C-17′), 20.0 (C-16′,20′), 22.8, 22.9 (C-18′,19′), 25.4 (C-1′), 26.5 (C-4), 25.1, 25.5, 26.1, 28.6, 33.3, 33.4, 37.4, 37.9, 38.0, 38.1, 40.0 (C-5′−C-15′), 40.8 (C-4′), 68.9 (C-3), 77.9 (C-2), 95.7 (C-8), 96.3 (C-6), 99.1 (C-4a), 106.7 (B-ring C-2.6), 110.4 (galloyl C-6), 121.1 (galloyl C-1), 124.1 (galloyl C-2), 124.7 (C-2′), 130.6 (B-ring C-1), 133.0 (B-ring C-4), 134.0 (C-3′), 137.3 (galloyl C-4), 142.8 (galloyl C-5), 144.7 (galloyl C-3), 146.1 (B-ring C-3,5), 156.9 (C-8a), 157.3 (C-5), 157.6 (C-7), 167.0 (galloyl C-17); MALDI TOF MS m/z 759 $[M + Na]^+$. Anal. Calcd for $C_{42}H_{56}O_{11}$: C, 68.46%; H, 7.66%. Found: C, 68.40%; H, 7.94%.

DPPH Radical Scavenging Activity of the Products. Each compound (0.1 μM) was partitioned between 20 mM phosphate buffer (pH 6.5, 750 μL) and n-octanol (750 μL) or triglyceride (750 μL, *Camellia* seed oil, 85% triolein) at 20 °C. After centrifugation, the organic layer (40 or 100 μL) was diluted to 1 mL by adding n-octanol. A portion (100 μL) of the diluted solution was added to a 0.2 mM ethanolic DPPH solution (50 μL) in a 96-well microplate and shaken for 30 min at 25 °C. The concentration of the DPPH radical was compared by measuring the absorbance at 490 nm. The scavenging activity (as a percentage) was expressed by [1 − (absorbance of the test sample solution/absorbance of the blank solution)] × 100. The partition coefficient between n-octanol and the phosphate buffer was measured by comparing UV absorptions of n-octanol and the aqueous layer at 280 nm after dilution (from 150 μL to 1.0 mL).

DPPH Radical Scavenging Activity of the Reaction Mixture. A solution of **2** (2 mg) in acetone (100 μL) was mixed with the test compound (15 μL) and dried under reduced pressure. The mixture was heated at 50 °C for 12 h and partitioned between *Camellia* seed oil

(triglyceride with a fatty acid composition of 85% oleic acid) and 20 mM sodium phosphate buffer (pH 6.5) (each 750 μL). After centrifugation, a portion of the organic layer (50 μL) was diluted to 1 mL by adding *n*-octanol. A portion (100 μL) of the diluted solution was used for comparing the radical scavenging activity. A control in the absence of **2** showed essentially no radical scavenging activity.

RESULTS AND DISCUSSION

The reactions of catechins with various aldehydes in foods or in vitro model experiments have been performed (*25−31*) and indicate that the initial step of the reaction is a simple electrophilic substitution at the C-8 and/or C-6 position(s) of the catechin A-ring. To develop a practical method to prepare catechin derivatives having hydrophobic alkyl moieties, we examined the condensation of catechins with three aldehydes [green leaf aldehyde (*trans*-2-hexenal), citral (3,7-dimethyl-2,6-octadien-1-al), and (+)-citronellal (3,7-dimethyloct-6-en-1-al)] and two allyl alcohols [geraniol (*trans*-3,7-dimethyl-2,6-octadien-1-ol) and phytol (*trans*-3,7,11,15-tetramethylhexadec-2-en-1-ol)] (**Figure 1**). In the present study, we propose a very simple reaction procedure (*29*) involving the mixture of catechin and aldehyde or alcohol heated or left at room temperature in the absence of solvent. Although the reactions gave mixtures of the products generated by nonselective condensation, the structures of the major products were elucidated.

Structures of the Products. Initially the reaction of (+)-catechin (**1**) with *trans*-2-hexenal was examined. The aldehyde is known as green leaf aldehyde and produced in the green tea leaf (*32*). The coupling reaction proceeded slowly at room temperature. After 1 week, TLC analysis indicated that the products were primarily less polar compared to **1**. However, purification of the less polar products was difficult because of the complex composition, and only compound **3** was isolated (**Figure 2**). MALDI TOF MS (*m*/*z* 683 $[M+Na]^+$) indicated that the molecular weight of this species was 660, which showed that two molecules of **1** were linked to a *trans*-2-hexenal unit in **3**. The ^{1}H and ^{13}C NMR and ^{1}H−^{1}H COSY spectra supported the molecular composition; however, the appearance of duplicate signals suggested the presence of two diastereomeric isomers differing in the configuration at the asymmetric centers (C-1″ and C-3″) of the hexane unit. HMBC correlations shown in **Figure 3** revealed that the C-8 carbons of both catechin units were substituted. The H-1″ of the hexane unit (δ 5.53 and 5.50, each dd, 2.5, 11.7 Hz) correlated to C-8 (δ 105.1, 105.5), C-8a (δ 153.3), and C-7 (δ 155.9, 156.0), indicating a C−C linkage between the C-8 and C-1″. In turn, the C-8′ correlated with the H-2″ methylene protons of the hexane unit. This correlation indicated that the C-3″ methine carbon was attached to the C-8′ of the other catechin unit. The molecular formula $C_{36}H_{36}O_{12}$ deduced from the molecular weight and elemental analysis indicated the degree of unsaturation of **3** was 19. This indicated the presence of an additional ring system besides the two catechin units. On the basis of the chemical shift of the C-1″ (δ 70.6, 71.5), the ring was determined to be formed by an ether linkage between the C-7′ hydroxyl group and the C-1″ of the hexane unit. The H-1″ of both diastereomeric isomers showed NOE correlations with the H-4″ methylene protons. Therefore, H-1″ and C-4″ were oriented to the same side of each molecule, and the relative configuration of the dihydropyran ring, including C-1″ and C-3″ of the isomers, was the same. Therefore, product **3** is a mixture of diastereomeric isomers differing in the absolute configuration of the C-1″ and C-3″ (**Figure 2**).

The reaction of epigallocatechin-3-*O*-gallate (**2**) and citral was subsequently examined. In addition to an α,β-unsaturated aldehyde structure, which is similar to the above-mentioned 2-hexenal, this aldehyde has an additional double bond at the C-6

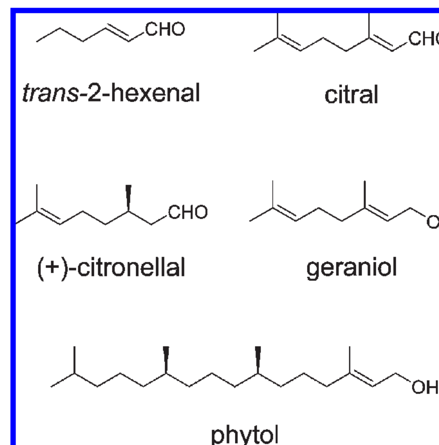

Figure 1. Structures of essential oils and phytol.

position. The reaction proceeded at 100 °C without solvent, and chromatographic separation of the products yielded four pure compounds, **4**−**7**.

The molecular formula of product **4** was shown to be $C_{32}H_{34}O_{12}$ on the basis of the $[M + Na]^+$ peak at *m*/*z* 633 and the $[M + K]^+$ peak at *m*/*z* 649 in the MALDI TOF MS and elemental analysis. The ^{1}H NMR spectrum showed signals as a result of the galloyl (δ 7.02, 2H, s), B-ring (δ 6.67, 2H, s), and C-ring (δ 5.49, br s, H-3; δ 5.15, br s, H-2; δ 3.09, br d; δ 2.95, dd, H-4) protons, which were similar to those of **2**. In addition, three singlet methyl signals at δ 0.85 (H-9′), 0.95 (H-8′), and 1.29 (H-10′) were observed. Besides signals arising from the unit of **2**, the ^{13}C NMR spectrum showed 10 aliphatic carbon signals including two oxygen-bearing quaternary carbons [δ 72.1 (C-7′), 75.0 (C-3′)]. In the HMBC spectrum, a methine proton signal resonating at δ 3.64 (H-1′) correlated to the C-3′ and three A-ring carbons (C-7, C-8, and C-8a) of the epigallocatechin unit. The two methylene (H-2′ and H-4′) and one methyl (H-10′) proton also correlated to the C-3′. The H-10′ methyl proton signal showed a long-range (^4J) coupling to the C-7 of the A-ring. These HMBC correlations indicated that C-1′ of the terpenoid unit was attached to C-8 of **2** and the C-3′ linked to the C-7 through an ether linkage. The presence of a terminal hydroxyisopropyl group (C-7′−C-9′) at C-6′ was revealed by the observation of HMBC correlations between H-6′ and C-7′ and between H-8′(9′) and C-6′. This C-6′ showed a correlation to the H-1′ and indicated the presence of a C−C bond between C-1′ and C-6′. This is consistent with the degree of unsaturation (16) calculated from the molecular formula. The result indicated that a menthane skeleton formed from the original citral molecule was attached to the C-8 and C-7 hydroxyl group in molecule **4**. As for the stereochemistry, the NOE correlation of the H-9′ to the H-2 and H-4β of the flavan C-ring (**Figure 3**) indicated that the configurations at the C-1′ and C-6′ were *S* and *R*, respectively (**Figure 2**).

Products **5** and **6** were shown to be isomers of **4** on the basis of the MALDI TOF MS, ^{1}H and ^{13}C NMR, and HMBC spectral data. The location of the menthane units at C-8 in **5** was confirmed by the appearance of HMBC correlations of the C-8 (δ 104.7) with H-1′ (δ 3.51) and H-2 (δ 4.99). The stereochemical difference between **4** and **5** was revealed by NOESY spectra, which showed NOE correlations of the hydroxyisopropyl methyl proton (H-9′) to the B-ring H-2(6) and galloyl H-2(6) protons. Thus, the configurations at the C-1′ and C-6′ of **5** were *R* and *S*, respectively. On the other hand, in the structure of **6**, the menthane unit was attached to the C-6. This is because both the C-ring H-2 (δ 5.03) and the A-ring singlet (δ 6.07, H-8) were correlated to the C-8a (δ 155.1) in the HMBC spectrum. In

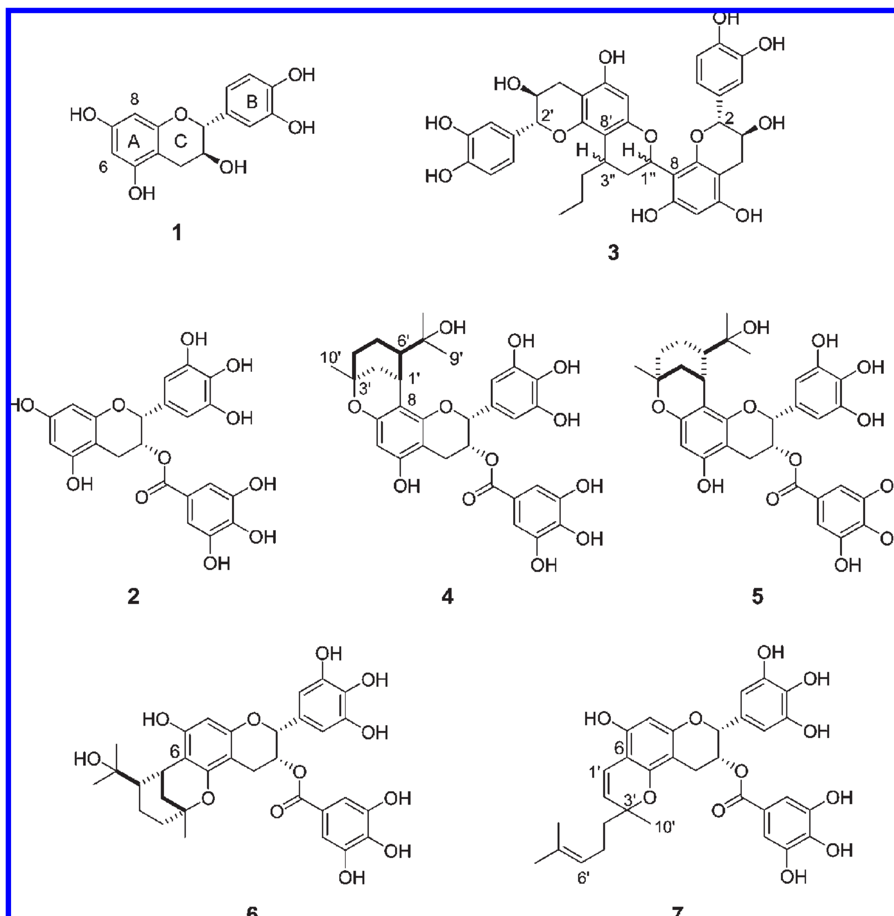

Figure 2. Structures of catechin (**1**), epigallocatechin-3-*O*-gallate (**2**), and products **3**−**7** obtained by coupling with *trans*-2-hexenal and citral.

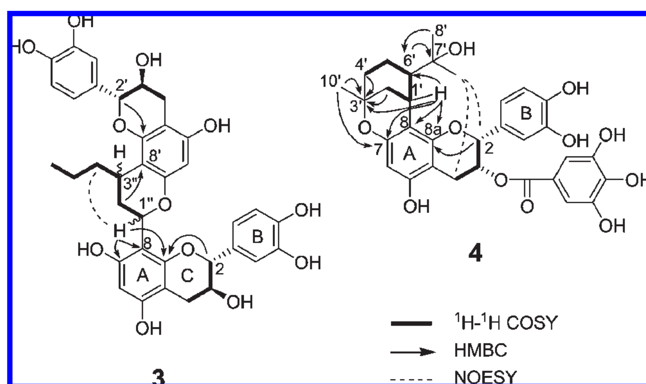

Figure 3. Important COSY, HMBC, and NOESY correlations for compounds **3** and **4**.

addition, the H-10′ methyl proton (*δ* 1.28) correlated to the C-5 (*δ* 155.8), which also correlated with the C-ring H-4 (*δ* 2.98). The configurations of the menthane C-1′ and C-6′ in **6** were deduced to be *R* and *S*, respectively, from the NOE correlation between the H-9 and galloyl H-2(6) protons (**Figure 2**).

The molecular formula of **7** was shown to be $C_{32}H_{32}O_{11}$ by MALDI TOF MS and elemental analysis. The HMBC correlations of the C-8a (*δ* 156.4) with the A-ring aromatic proton (*δ* 6.07, H-8) and C-ring H-2 (*δ* 5.05) revealed a substitution at the C-6 of the epigallocatechin unit. The carbon signals arising from the terpenoid unit were related to those of citral. The ^{1}H−^{1}H COSY spectrum indicated the presence of a 4-methylpent-3-enyl moiety (C-4′−C-9′). In addition, signals attributable to a cis-double bond (*δ* 6.63, 5.41, each d, J = 10.0 Hz, H-1′ and H-2′, respectively) were observed. Both of these olefinic protons were correlated to the C-6 in the HMBC spectrum. Furthermore, the H-1′ (*δ* 6.63) showed a correlation peak with the C-5, which was unambiguously assigned by correlation with the C-ring H-4. These olefinic protons and the C-4′ and C-5′ methylene protons showed HMBC correlations with an oxygen-bearing quaternary carbon (*δ* 79.1, C-3′). The degree of unsaturation (17) indicated that the C-3′ forms a pyran ring with the C-5 or C-7 phenolic hydroxyl group. The location of the pyran ring was determined to be C-5 by observation of the ^4J HMBC correlation between the C-5 and C-10′ methyl protons. Thus, the arrangement of this product is represented by structure **7**.

Unlike two previous α,*β*-unsaturated aldehydes, (+)-citronellal does not have a conjugated structure. Two coupling products, **8** and **9**, were isolated from the reaction mixture with **2**. MALDI TOF MS analysis indicated that two citronellal units were substituted in **8** (*m*/*z* 753 $[M + Na]^+$), and one unit was substituted in **9** (*m*/*z* 617 $[M + Na]^+$). Both of the products were shown to be a mixture of diastereomeric isomers by ^{1}H and ^{13}C NMR spectroscopic analysis. That is, **9** was a mixture of two isomers and **8** was composed of four diastereomers. Comparison of ^{1}H and ^{13}C NMR chemical shifts with a model compound **10** prepared from phloroglucinol and citronellal (*33*) enabled us to deduce the structures of **8** and **9** (**Figure 4**). The location of the menthane moiety in **9** was determined by the observation of HMBC correlations between H-8′ and C-7 (^4J correlation) and between H-1′ and C-8 (*δ* 105.9 and 107.6). The assignment of the A-ring carbons was confirmed by HMBC correlations of H-2 with C-8a and H-6 with C-5 and C-7.

Geraniol, an essential oil with an allyl alcohol structure, reacted with **2** under acidic conditions. A mixture of geraniol

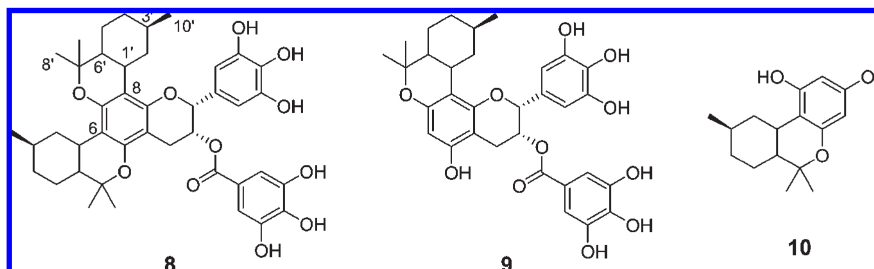

Figure 4. Structures of compounds **8**−**10** obtained by coupling with citronellal.

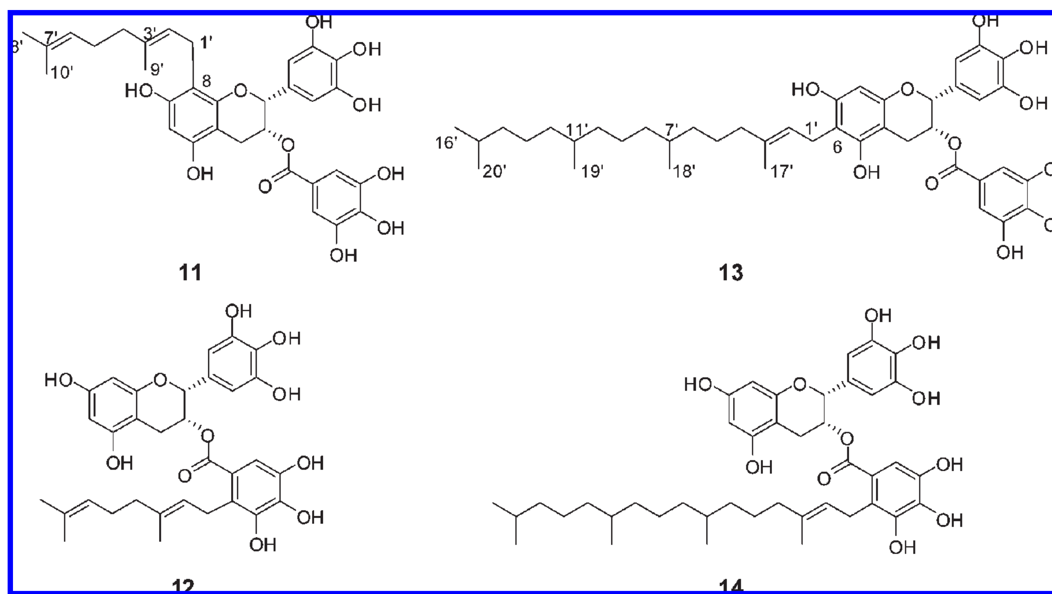

Figure 5. Structures of compounds **11**−**14** obtained by coupling with geraniol and phytol.

and **2** was heated in the presence of citric acid, and two products, **11** and **12**, were isolated (see **Figure 5**). Both compounds showed an $[M + Na]^+$ peak at m/z 617, indicating the same molecular constitution. The ^{1}H and ^{13}C NMR spectra were also related to each other. Comparison of the ^{13}C NMR chemical shifts with those of geraniol revealed a 3,7-dimethylocta-2,6-dienenyl (geranyl) group in each molecule. On the basis of the appearance of the HMBC correlation between H-1′ and C-8, the location of the geranyl group in **11** was shown to be at C-8. The assignment of the C-8 carbon signal was based on the observation of correlations between the A-ring carbons and H-2 (to C-8a), H-4 (to C-4a, C-5, and C-8a), and H-6 (to C-4a, C-5, C-7, and C-8). In contrast, the geranyl moiety in **12** was determined to be attached at the C-2 of the galloyl group. Two doublet signals (J = 2.3 Hz) of the A-ring H-6 and H-8 were observed at δ 6.04 and 5.98, respectively. In contrast, the signal of the galloyl group was observed as a one-proton singlet at δ 7.01. The appearance of six aromatic carbon signals was attributed to the galloyl moiety in the ^{13}C NMR spectrum, indicating the presence of asymmetrical substitutions. In addition, one of the galloyl carbons (δ 124.1, C-2) showed an HMBC correlation with the H-1′ methylene protons of the geraniol moiety. Thus, the conformation was represented by structure **12**.

Phytol, which is a component of chlorophylls, has the same partial structure as geraniol. Reaction with **2** under acidic conditions (at 50 °C for 30 h in the presence of HCl) gave two products, **13** and **14** (see **Figure 5**). MALDI TOF MS of these products (m/z 759 $[M + Na]^+$) indicated that one molecule of phytol was attached to molecule **2** in these products. The ^{1}H NMR signals of **13** were related to those of **11**, except for the appearance of complex signals arising from the phytol moiety. The A-ring singlet δ 6.13 was assigned to the H-8 proton, because this signal and the C-ring H-2 showed ^3J HMBC correlations to the C-8a (δ 154.2). Furthermore, the C-1′ methylene protons of the phytol moiety were correlated with A-ring C-5 (δ 154.7), C-6 (δ 108.9), and C-7 (δ 155.0). These HMBC correlations revealed that the phytol C-1 was attached to the C-6 of the flavan-3-ol moiety. On the other hand, the ^{1}H NMR spectrum of **14** was related to that of **12**: the appearance of mutually coupled A-ring proton signals (δ 5.98 and 6.04, d, J = 2.3 Hz) and the one-proton singlet at δ 6.92, which was assigned to the galloyl proton, indicated the location of the phytol moiety to be at the galloyl C-2 position. This was consistent with the HMBC correlations between the galloyl C-2 (δ 124.1) and the phytol H-1′ protons [δ 3.56 (dd, J = 6.6, 14.0 Hz), 3.64 (dd, J = 6.6, 14.0 Hz)].

DPPH Radical Scavenging Activity. The products obtained in this study were expected to show higher lipid solubility when compared to the lipid solubility of **2**. Therefore, DPPH radical scavenging activities of the organic layer after partitioning with phosphate buffer (pH 6.5) were compared. In the presence of *n*-octanol, the octanol layers of all products showed similar radical scavenging activities when compared to the activity of **2** (**Table 1**). However, when *Camellia* seed oil (triglyceride with a fatty acid composition of 85% oleic acid) was used, the activity of the organic layer of **2** was very small. In contrast, organic layers of the products **6**−**9**, **11**, and **13** showed relatively higher activities. Each reaction of catechin with the essential oil gave a complex mixture, and the products isolated in this study may not represent the total reaction products. Therefore, the lipid-soluble fractions of the reaction mixtures of **2** with various aldehydes (*trans*-2-hexenal, citral, citronellal, perillaldehyde, cinnamaldehyde, and *trans*-2-nonenal),

Table 1. DPPH Radical Scavenging Activity of the Products after Solvent Partitioning

	scavenging activity (%)			
	n-octanol layer	triglyceride layer		
product	40 μL[a]	100 μL[a]	40 μL[a]	log $P_{octanol}$
2	32.6 ± 5.6	1.2 ± 6.6	2.9 ± 5.2	1.00
4	29.9 ± 3.6	9.5 ± 5.7	3.7 ± 4.8	1.85
6	32.0 ± 0.7	59.3 ± 6.3	38.5 ± 2.3	2.66
7	23.4 ± 2.0	51.9 ± 7.9	26.4 ± 2.8	2.72
8	30.0 ± 2.1	68.5 ± 4.9	42.3 ± 3.6	>10
9	28.7 ± 3.1	47.9 ± 8.5	17.1 ± 2.1	2.03
11	44.3 ± 3.7	80.1 ± 0.9	66.7 ± 3.6	2.55
13	41.8 ± 0.5	78.3 ± 0.9	68.8 ± 4.7	>10

[a] A potion (40 or 100 μL) of organic layer (750 μL) after partition was diluted to 1.0 mL, and 100 μL of the diluted solution was added to 0.2 mM ethanolic DPPH solution (50 μL).

Table 2. DPPH Radical Scavenging Activity of Triglyceride Layer after Partitioning of the Reaction Mixture with Triglyceride/Water

	scavenging activity (%)
2	7.7 ± 3.9
2 + *trans* −2-hexenal	0.9 ± 4.1
2 + citral	48.1 ± 3.9
2 + citronellal	88.4 ± 0.6
2 + geraniol	85.6 ± 0.6
2 + phytol	89.5 ± 0.0
2 + perillaldehyde	38.5 ± 2.6
2 + cinnnamaldehyde	68.7 ± 1.3
2 + 3-nonen-2-one	36.3 ± 4.0
2 + *trans*-2-nonenal	87.1 ± 0.4
2 + lemon grass essential oil	71.1 ± 1.5

alcohols (geraniol and phytol), a ketone (3-nonen-2-one), and commercial lemongrass essential oil (major component is citral) were compared (**Table 2**). Except for *trans*-2-hexenal, the triglyceride-soluble fractions of the reaction mixtures showed strong radical scavenging activities compared to the activity of **2**.

In this study, we introduced a simple method for the preparation of coupling products of catechin and aldehydes and allyl alcohols. The reactions resulted in the production of complex mixtures resulting from the generation of positional and diastereomeric isomers. Therefore, the practical application of the condensation products as functional materials may be limited in the form of a mixture of the isomers. Similar reactions may occur during food processing, such as the heating of tea leaves, because aldehydes are generated from unsaturated fatty acids by oxidative degradation. The chemical modification of polyphenol molecules is an interesting subject in food and medicinal sciences because of the attractive biological activities of catechins. In addition to the increase of DPPH radical scavenging activity in hydrophobic conditions, a preliminary study on the antibacterial activity of the products by the disk diffusion method (*34*) suggests that compounds **11** and **12** show stronger activities than compound **2** against *Staphylococcus aureus*. Further studies on biological activities are currently in progress.

ACKNOWLEDGMENT

We are grateful to K. Inada, N. Yamaguchi, and N. Tsuda, Nagasaki University Joint Research Center, for NMR and MS measurements and elemental analysis.

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Received April 2, 2009. Revised manuscript received May 18, 2009. Accepted May 20, 2009. This work was supported by Grant-in-aid for Scientific Research 18510189 from the Japan Society for the Promotion of Science.