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Antioxidative Compounds from the Outer Scales of Onion

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Antioxidative compounds were isolated from the methanol extract of dry outer scales of onion (*Allium cepa* L.). Nine phenolic compounds (**1**−**9**) were finally obtained by reversed-phase high-performance liquid chromatography, and their structures were elucidated by NMR and mass spectrometry analyses. They were the six known compounds, protocatechuic acid (**1**), 2-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxy-3(2*H*)-benzofuranone (**2**), quercetin 4′-*O*-*β*-D-glucopyranoside (**3**), quercetin (**5**), 4′-*O*-*β*-D-glucopyranoside of quercetin dimer (**7**), and quercetin dimer (**8**), and three novel compounds, condensation products of quercetin with protocatechuic acid (**4**), adduct of quercetin with quercetin 4′-*O*-*β*-D-glucopyranoside (**6**), and quercetin trimer (**9**). These phenolic compounds were tested for their antioxidant properties using autoxidation of methyl linoleate in bulk phase or free radical initiated peroxidation of soybean phosphatidylcholine in liposomes. The flavonoid compounds having *o*-dihydroxy substituent in the B-ring were shown to be effective antioxidants against nonenzymic lipid peroxidation.

KEYWORDS: antioxidant; flavonoid; quercetin; lipid peroxidation; onion; *Allium cepa*

INTRODUCTION

High dietary intakes of fruits and vegetables have been reported to correlate with a low risk of degenerative diseases from epidemiological evidence (*1*). The protective effects have been attributed partly to the various antioxidative compounds present in fruits and vegetables (*2*). The most abundant types of antioxidative compounds in human diet are flavonoids, which are present in plant tissues in relatively high concentrations, either as sugar conjugates or as aglycones (*3*, *4*). Onion (*Allium cepa* L.) is one of the major sources of dietary flavonoids in many countries. It shows a variety of pharmacological effects such as growth inhibition of tumor and microbial cells, reduction of cancer risk, scavenging of free radicals, and protection against cardiovascular disease, which are attributed to specific sulfur-containing compounds and flavonoids (*5*, *6*). The major flavonoid found in onion is quercetin, present as quercetin 4′-*O*-*β*-glucopyranoside, quercetin 3,4′-*O*-*β*-diglucopyranoside, and quercetin 3,7,4′-*O*-*β*-triglucopyranoside (*7*−*9*). Regardless of high levels of flavonoids in outer scales of onion, they are peeled off and wasted before food processing such as cooking. The outer scales contain some quercetin derivatives (*10*−*12*). Takahama and Hirota (*10*) have suggested that quercetin is formed by the deglucosidation of its glucosides, followed by autoxidation to produce protocatechuic acid. Recently, some anti-platelet and membrane-rigidifying flavonoids have been isolated from outer scales of onion and identified as quercetin, quercetin dimers, and quercetin 4′-glucoside (*11*, *12*). Although extracts from the onion outer scales have exhibited potent radical scavenging activities (*13*, *14*), the specific antioxidative components are still unclear.

This study undertook the isolation and structural elucidation of antioxidative compounds from the dry outer scales of onion bulb. 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 liposomal systems.

MATERIALS AND METHODS

Materials. Dry outer scales of yellow onion bulb were obtained from Ibiden Products Co., Ltd. (Gifu, Japan). *RRR*-α-Tocopherol (Sigma Chemical Co., St. Louis, MO) was purified by high-performance liquid chromatography (HPLC) before use (*15*). Methyl linoleate (Tokyo Chemical Co., Tokyo, Japan) was purified by silica gel column chromatography to be peroxide free (*16*). Soybean L-α-PC was purchased from Avanti Polar Lipid, Inc. (Alabaster, AL) and purified by silica gel column chromatography with chloroform/methanol as the solvent. Methyl linoleate hydroperoxides and PC hydroperoxides (PC−OOH) were prepared as described previously (*17*, *18*). 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

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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 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. High-resolution fast-atom bombardment mass (HR-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) equipped with ESI and APCI sources, respectively. Sample was delivered into the ion source using methanol/water (95:5, v/v) containing 0.2% acetic acid at 0.1 mL/min.

Extraction and Isolation Procedures. Dry outer scales of onion (100 g) were successively extracted three times with hot methanol (1 L) for 2 h. After the solvent was removed, the methanol extract (10.3 g) was subjected to chromatography on a 20 × 6.0 cm inside diameter (i.d.) Amberlite XAD-2 resin column. The column was eluted with distilled water (2 L) to eliminate water-soluble compounds, and the retained material was eluted with methanol (2 L). The methanol eluate, which had antioxidative activity with 90% inhibition against methyl linoleate autoxidation in bulk phase after a 36-h incubation, was concentrated to dryness in vacuo to afford the methanol fraction (5.10 g).

The methanol fraction (5.10 g) was subjected to chromatography on a 15 × 3.4 cm i.d. 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 (80:20:1, 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. Fractions with the same TLC patterns were pooled to give fraction I (1.11 g) obtained from the elution of chloroform/methanol (90:10, v/v), fractions II (0.87 g) and III (0.25 g) from the elution of chloroform/methanol (85:15, v/v), and fraction IV (1.17 g) from the elution of chloroform/methanol (80:20, v/v), respectively. Fractions I−IV were further purified by HPLC. Reversed-phase HPLC was done with a 250 × 10 mm i.d. Inertsil PREP-ODS column developed with 0.1% acetic acid in acetonitrile/water (40:60, v/v; for fraction I), acetonitrile/water (35:65, v/v; for fractions II and IV), or acetonitrile/water (30:70, v/v; for fraction III) at a flow rate of 5.0 mL/min. The eluate was monitored by absorbance at 280 nm. Compounds **1** (102.7 mg) and **5** (314.6 mg) were isolated from fraction I, and compounds **4a** (15.7 mg), **4b** (23.3 mg), **8** (86.8 mg), and **9** (32.7 mg) were isolated from fraction II, compound **2** (18.5 mg) was from fraction III, and compounds **3** (83.9 mg), **6** (45.7 mg), and **7** (52.6 mg) were from fraction IV.

HPLC Analysis. HPLC was carried out using a Shimadzu LC-10AV*vp* pump equipped with a Shimadzu SPD-10A*vp* UV−Vis detector (Shimadzu Co., Kyoto, Japan). The methanol extract of outer scales of onion was separated on a 100 × 2.0 mm i.d. Hydrosphere C18 column (YMC Co., Ltd.; Kyoto, Japan) at 40 °C. The mobile phase consisted of 70% acetonitrile with 0.2% formic acid (A) and 20% acetonitrile with 0.2% formic acid (B). The linear gradient was 0−100% (A) in 20 min, at a flow rate 0.2 mL/min. Compounds were detected by monitoring the elution at 280 nm.

Derivatization. To a methanol solution of compound **4a**, **4b**, **6**, or **9**, an excess of diazomethane in ether was added, and the reaction mixture was allowed to stir overnight. Each crude reaction mixture was then purified by silica gel column (eluted by increasing methanol concentration in mixtures of chloroform and methanol). The methylated **6** was hydrolyzed, and the resulting aglycone was acetylated with acetic anhydride in pyridine.

Structures of Compounds 1−9. *3,4-Dihydroxybenzoic Acid (protocatechuic acid, **1**).* Brown amorphous solid; UV (methanol) λ_{max} (log ϵ) 257 (4.03) and 294 nm (3.80); HR-FABMS *m/z* 154.0253 (M^+), calcd for $C_7H_6O_4$, 154.0266; ESIMS (negative) *m/z* 153.00 ($[M - H]^-$, 100%); APCIMS (negative) *m/z* 153.00 ($[M - H]^-$, 100%) and 307.00 ($[2M - H]^-$, 70%).

*2-(3,4-Dihydroxybenzoyl)-2,4,6-trihydroxy-3(2H)-benzofuranone (**2**).* Brown-red amorphous solid; $[\alpha]^{24}_D$ +5.5 (*c* 0.49, methanol); UV (methanol) λ_{max} (log ϵ) 291 nm (4.39); HR-FABMS *m/z* 319.0441 ($[M + H]^+$), calcd for $C_{15}H_{11}O_8$, 319.0454; ESIMS (positive) *m/z* 340.95 ($[M + Na]^+$, 100%) and 319.00 ($[M + H]^+$, 10%); ESIMS (negative) *m/z* 317.00 ($[M - H]^-$, 100%); APCIMS (negative), *m/z* 317.00 ($[M - H]^-$, 100%); ^{1}H NMR (CD_3OD) δ 5.97 (s, 1H, H-6), 6.00 (s, 1H, H-8), 6.79 (d, J = 8.0 Hz, 1H, H-5′), 7.59 (dd, J = 2.3, 8.0 Hz, 1H, H-6′), and 7.62 (d, J = 2.3 Hz, 1H, H-2′); ^{13}C NMR (CD_3OD) δ 92.1 (C-8), 97.9 (C-6), 102.0 (C-4a), 105.6 (C-3), 115.6 (C-5′), 118.2 (C-2′), 125.4 (C-6′), 126.6 (C-1′), 146.1 (C-3′), 152.9 (C-4′), 160.4 (C-8a), 171.2 (C-5), 174.0 (C-7), 191.7 (C-4), and 193.4 (C-2).

2-[4-(*β*-D-*Glucopyranosyloxy)-3-hydroxyphenyl]-3,5,7-trihydroxy-4H-1-benzopyran-4-one (quercetin 4′-O-β-D-glucopyranoside, **3**).* Yellow amorphous solid; $[\alpha]^{24}_D$ −65.5 (*c* 0.40, methanol); UV (methanol) λ_{max} (log ϵ) 253 (4.37) and 364 nm (4.34); HR-FABMS *m/z* 465.0985 ($[M + H]^+$), calcd for $C_{21}H_{21}O_{12}$, 465.1033; ESIMS (positive) *m/z* 487.10 ($[M + Na]^+$, 60%) and 465.20 ($[M + H]^+$, 100%); ESIMS (negative) *m/z* 463.05 ($[M - H]^-$, 100%); APCIMS (negative), *m/z* 463.00 ($[M - H]^-$, 100%) and 301.05 ($[C_{15}H_9O_7]^-$, 30%); ^{1}H NMR(CD_3OD) δ 3.47 (m, 1H, H-4″), 3.50 (m, 1H, H-5″), 3.55 (m, 1H, H-3″), 3.56 (m, 1H, H-2″), 3.76 (dd, J = 5.2, 12.0 Hz, 1H, H-6″a), 3.95 (dd, J = 2.3, 12.0 Hz, 1H, H-6″b), 4.92 (d, J = 6.9 Hz, 1H, H-1″), 6.15 (s, 1H, H-6), 6.33 (s, 1H, H-8), 7.25 (d, J = 8.6 Hz, 1H, H-5′), 7.65 (d, J = 8.6 Hz, 1H, H-6′), and 7.71 (s, 1H, H-2′); ^{13}C NMR (CD_3OD) δ 62.5 (C-6″), 71.4 (C-4″), 74.9 (C-2″), 77.6 (C-5″), 78.4 (C-3″), 94.5 (C-8), 99.4 (C-6), 103.5 (C-1″), 104.6 (C-4a), 116.5 (C-2′), 117.6 (C-5′), 121.3 (C-6′), 127.6 (C-1′), 137.9 (C-3), 146.8 (C-3′), 147.8 (C-2), 148.1 (C-4′), 158.2 (C-8a), 162.5 (C-5), 165.7 (C-7), and 177.4 (C-4).

*Compounds **4a** and **4b**.* **4a**: Brown-red amorphous solid; $[\alpha]^{24}_D$ +14.01 (*c* 0.16, methanol); UV (methanol) λ_{max} (log ϵ) 291 nm (3.47); HR-FABMS *m/z* 455.0652 ($[M + H]^+$), calcd for $C_{22}H_{15}O_{11}$, 455.0614; ESIMS (negative) *m/z* 453.05 ($[M - H]^-$, 100%) and 299.05 ($[C_{15}H_7O_7]^-$, 90%); APCIMS (negative) *m/z* 453.25 ($[M - H]^-$, 5%), 301.00 ($[C_{15}H_9O_7]^-$, 100%); ^{1}H NMR (CD_3OD) δ 5.94 (s, 2H, H-6, H-8), 6.69 (d, J = 8.6 Hz, 1H, H-5′), 7.05 (dd, J = 2.3, 8.6 Hz, 1H, H-6′), 7.11 (d, J = 8.6 Hz, 1H, H-5*′), 7.23 (d, J = 2.3 Hz, 1H, H-2′), 7.60 (d, J = 1.7 Hz, 1H, H-2*′), and 7.70 (dd, J = 2.3, 8.6 Hz, 1H, H-6*′); ^{13}C NMR (CD_3OD) δ 91.8 (C-3), 97.4 (C-8), 98.0 (C-6), 101.3 (C-4a), 102.0 (C-2), 115.5 (C-5′), 116.8 (C-2′), 118.1 (C-2*′), 120.2 (C-5*′), 121.3 (C-6′), 125.6 (C-6*′), 126.7 (C-1′), 127.1 (C-1*′), 142.1 (C-3*′), 145.8 (C-3′), 146.1 (C-4*′), 148.1 (C-4′), 161.1 (C-8a), 165.4 (C-5), 169.4 (*C*OOH), 169.7 (C-7), and 189.8 (C-4) (tentative assignments based on ^{1}H-^{1}H COSY, HMBC, and HMQC). Hexamethyl derivative of **4a**: APCIMS (positive) *m/z* 539.25 ($[M + H]^+$, 100%); ^{1}H NMR ($CDCl_3$) δ 3.74 (s, 3H, 3-OC*H*$_3$), 3.81 (s, 3H, 7-OC*H*$_3$), 3.842 (s, 3H, 3′-OC*H*$_3$), 3.844 (s, 3H, 4′-OC*H*$_3$), 3.87 (s, 3H, 5-OC*H*$_3$), 3.88 (s, 3H, COOC*H*$_3$), 6.10 (s, 1H, H-6), 6.13 (s, 1H, H-8), 6.77 (d, J = 8.6 Hz, 1H, H-5′), 7.10 (dd, J = 8.6 Hz, 1H, H-5*′), 7.26 (d, J = 6.9 Hz, 1H, H-6′), 7.32 (s, 1H, H-2′), 7.73 (d, J = 8.6 Hz, 1H, H-6*′), and 7.75 (d, J = 1.7 Hz, 1H, H-2*′). **4b**: Brown-red amorphous solid; $[\alpha]$ $^{24}_D$ +46.4 (*c* 0.23, methanol); UV (methanol) λ_{max} (log ϵ) 290 nm (3.54); HR-FABMS *m/z* 455.0581 ($[M + H]^+$), calcd for $C_{22}H_{15}O_{11}$, 455.0614; ESIMS (negative) *m/z* 453.00 ($[M - H]^-$, 80%), 299.05 ($[C_{15}H_7O_7]^-$, 100%); APCIMS (negative) *m/z* 453.25 ($[M - H]^-$, 5%) and 301.05 ($[C_{15}H_9O_7]^-$, 100%); ^{1}H NMR (CD_3OD) δ 5.94 (s, 2H, H-6, H-8), 6.69 (d, J = 8.6 Hz, 1H, H-5′), 7.02 (d, J = 8.6 Hz, 1H, H-5*′), 7.05 (dd, J = 2.3, 8.6 Hz, 1H, H-6′), 7.23 (d, J = 2.3 Hz, 1H, H-2′), 7.68 (d, J = 8.6 Hz, 1H, H-6*′), and 7.69 (s, 1H, H-2*′); ^{13}C NMR (CD_3OD) δ 92.1 (C-3), 97.4 (C-8), 98.0 (C-6), 101.3 (C-4a), 101.8 (C-2), 115.5 (C-5′), 116.8 (C-2′), 118.4 (C-2*′), 119.9 (C-5*′), 121.3 (C-6′), 125.8 (C-6*′), 126.68 (C-1′), 126.71 (C-1*′), 141.7 (C-3*′), 145.8 (C-3′), 146.6 (C-4*′), 148.1 (C-4′), 161.1 (C-8a), 165.4 (C-5), 169.4 (*C*OOH), 169.7 (C-7), and 189.6 (C-4) (tentative assignments

based on ^{1}H-^{1}H COSY, HMBC, and HMQC). Hexamethyl derivative of **4b**: APCIMS (positive) *m/z* 539.25 ([M + H]$^+$, 100%); ^{1}H NMR ($CDCl_3$) δ 3.73 (s, 3H, 3-OC*H*$_3$), 3.81 (s, 3H, 7-OC*H*$_3$), 3.84 (s, 3H, 3′-OC*H*$_3$), 3.85 (s, 3H, 4′-OC*H*$_3$), 3.87 (s, 3H, 5-OC*H*$_3$), 3.89 (s, 3H, COOC*H*$_3$), 6.10 (s, 1H, H-6), 6.13 (s, 1H, H-8), 6.77 (d, J = 8.6 Hz, 1H, H-5′), 7.08 (dd, J = 8.6 Hz, 1H, H-5*′), 7.25 (d, J = 6.9 Hz, 1H, H-6′), 7.33 (s, 1H, H-2′), 7.71 (d, J = 8.6 Hz, 1H, H-6*′), and 7.78 (d, J = 1.7 Hz, 1H, H-2*′).

*2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one (quercetin, **5**).* Yellow amorphous solid; UV (methanol) λ_{max} (log ϵ) 255 (4.36) and 371 nm (4.33); HR-FABMS *m/z* 303.0508 ([M + H]$^+$), calcd for $C_{15}H_{11}O_7$, 303.0505; ESIMS (positive) *m/z* 303.05 ([M + H]$^+$, 100%); ESIMS (negative) *m/z* 301.05 ([M − H]$^-$, 100%); APCIMS (negative) *m/z* 301.05 ([M − H]$^-$, 100%).

*Compound **6**.* Brown-red amorphous solid; UV (methanol) λ_{max} (log ϵ) 254 (4.50), 304 (4.46), and 371 (4.34); HR-FABMS *m/z* 765.1339 ([M + H]$^+$), calcd for $C_{36}H_{29}O_{19}$, 765.1303; ESIMS (positive) *m/z* 787.15 ([M + Na]$^+$, 40%), 765.15 ([M + H]$^+$, 100), ESIMS (negative) *m/z* 763.05 ([M − H]$^-$, 100%); APCIMS (negative) *m/z* 763.35 ([M − H]$^-$, 4%), 611.00 ([$C_{29}H_{23}O_{15}$]$^-$, 100%); ^{1}H NMR (CD_3OD) δ 3.43 (m, 1H, H-4″), 3.54 (m, 3H, H-2″, H-3″, H-5″), 3.77 (dd, J = 5.7, 12.0 Hz, 2/ 3H, H-6″a), 3.79 (dd, J = 6.3, 12.0 Hz, 1/3H, H-6″a), 4.04 (dd, J = 2.3, 12.0 Hz, 1/3H, H-6″b), 4.05 (dd, J = 2.3, 12.0 Hz, 2/3H, H-6″b), 4.85 (d, J = 7.4 Hz, 1/3H, H-1″), 4.87 (d, J = 7.4 Hz, 2/3H, H-1″), 5.98 (s, 1H, H-6*), 6.03 (s, 1H, H-8*), 6.25 (dd, J = 2.3, 8.6 Hz, 1/3H, H-6′), 6.33 (dd, J = 2.3, 8.6 Hz, 2/3H, H-6′), 6.42 (s, 1H, H-6), 6.816 (d, J = 8.6 Hz, 1/3H, H-5*′), 6.820 (d, J = 8.6 Hz, 2/3H, H-5*′), 6.86 (dd, J = 2.3, 8.6 Hz, 1/3H, H-6*′), 6.87 (dd, J = 2.3, 8.0 Hz, 2/3H, H-6*′), 6.97 (d, J = 1.7 Hz, 2/3H, H-2*′), 6.99 (d, J = 1.7 Hz, 1/3H, H-2*′), 7.00 (d, J = 8.6 Hz, 2/3H, H-5′), 7.04 (d, J = 8.6 Hz, 1/3H, H-5′), 7.76 (d, J = 1.7 Hz, 2/3H, H-2′), and 7.78 (d, J = 2.3 Hz, 1/3H, H-2′). ^{13}C NMR (CD_3OD) δ 62.6 and 62.7 (C-6″), 71.5 and 71.6 (C-4″), 74.8 (C-2″), 77.58 and 77.63 (C-5″), 78.3 and 78.5 (C-3″), 93.67 and 93.71 (C-2*), 95.6 (C-6), 96.4 (C-8*), 97.8 (C-6*), 100.3 (C-4a*), 103.2 and 103.7 (C-1″), 104.4 (C-3*), 106.3 (C-4a), 108.7 and 108.8 (C-8), 116.0 and 116.1 (C-5*′), 116.9 and 117.0 (C-2*′), 117.25 and 117.35 (C-5′), 117.35 and 117.44 (C-2′), 120.69 and 120.74 (C-6*′), 120.86 and 120.91 (C-6′), 126.9 (C-1′), 127.2 (C-1*′), 138.2 (C-3), 146.17 and 146.22 (C-3*′), 146.93 and 146.98 (C-2), 146.99 and 147.04 (C-4*′), 147.3 and 147.4 (C-3′), 148.2 and 148.4 (C-4′), 153.2 (C-8a), 164.3 (C-8a*), 165.7 (C-5), 166.5 (C-5*), 167.59 and 167.64 (C-7), 170.5 (C-7*), 177.7 (C-4), and 186.9 (C-4*). The sugar composition was determined to be glucose by GLC as an alditol acetate after hydrolysis. 4′-*O*-Acetyl-octamethyl derivative of the aglycone (**6′**): APCIMS (positive) *m/z* 757.30 ([M + H]$^+$, 5%), 637.30 ([M − 119]$^+$, 100%); ^{1}H NMR ($CDCl_3$) δ 1.26 (s, 3H, COOC*H*$_3$), 3.71 (s, 3H, OC*H*$_3$), 3.79 (s, 3H, OC*H*$_3$), 3.80 (s, 3H, OC*H*$_3$), 3.81 (s, 3H, OC*H*$_3$), 3.82 (s, 3H, OC*H*$_3$), 3.84 (s, 3H, OC*H*$_3$), 3.88 (s, 3H, OC*H*$_3$), 3.93 (s, 3H, OC*H*$_3$), 6.14 (s, 1H, H-6*), 6.15 (s, 1H, H-8*), 6.70 (dd, J = 2.3, 8.6 Hz, 1/3H, H-6*′), 6.74 (dd, J = 2.3, 8.6 Hz, 2/3H, H-6*′), 6.77 (d, J = 8.6 Hz, 1/3H, H-5*′), 6.78 (s, 2/3H, H-2*′), 6.788 (s, 1H, H-6), 6.793 (d, J = 8.0 Hz, 2/3H, H-5*′), 6.81 (s, 1/3H, H-2*′), 7.08 (d, J = 10.9 Hz, 2/3H, H-5′), 7.10 (d, J = 8.0 Hz, 1/3H, H-5′), 7.50 (s, 1/2H, H-2′), 7.52 (s, 1/2H, H-2′), 7.66 (dd, J = 2.3, 8.6 Hz, 1/3H, H-6′), and 7.69 (dd, J = 2.3, 8.6 Hz, 2/3H, H-6′).

*1,3,11a-Trihydroxy-9-(3,5,7-trihydroxy-4H-1-benzopyran-4-on-2-yl)-5a-[4-(β-D-glucopyranosyloxy)-3-hydroxyphenyl]-5,6,11-hexahydro-5,6,11-trioxanaphthacene-12-one (**7**).* Brown-red amorphous solid; UV (methanol) λ_{max} (log ϵ) 269 (4.45), 301 (4.51), and 360 nm (4.41); HR-FABMS *m/z* 765.1367 ([M + H]$^+$), calcd for $C_{36}H_{29}O_{19}$, 765.1303; ESIMS (positive) *m/z* 787.25 ([M + Na]$^+$, 100%); ESIMS (negative) *m/z* 763.10 ([M − H]$^-$, 85%) and 299.00 ([$C_{15}H_{10}O_7$]$^-$, 100%); ^{1}H NMR (CD_3OD) δ 3.40 (m, 1H, H-4″), 3.43 (m, 1H, H-5″), 3.48 (m, 1H, H-3″), 3.50 (m, 1H, H-2″), 3.70 (dd, J = 5.2, 12.0 Hz, 1H, H-6″a), 3.93 (d, J = 12.0 Hz, 1H, H-6″b), 4.84 (d, J = 6.9 Hz, 1H, H-1″), 5.96 (s, 1H, H-6), 5.98 (s, 1H, H-8), 6.18 (s, 1H, H-6*), 6.39 (s, 1H, H-8*), 7.14 (d, J = 8.6 Hz, 1H, H-5′), 7.18 (m, 1H, H-6′), 7.19 (d, J = 8.6 Hz, 1H, H-5*′), 7.328 (d, J = 2.9 Hz, 1/2H, H-2′), 7.333 (d, J = 2.3 Hz, 1/ 2H, H-2′), 7.87 (d, J = 8.6 Hz, 1H, H-6*′), and 7.88 (s, 1H, H-2*′); ^{13}C NMR (CD_3OD) δ 62.4 (C-6″), 71.3 (C-4″), 74.8 (C-2″), 77.5 (C-5″), 78.2 (C-3″), 91.8 (C-3), 94.6 (C-8*), 97.4 (C-8), 98.1 (C-6), 99.4 (C-6*), 101.2 (C-4a), 101.7 (C-2), 103.4 (C-1″), 104.6 (C-4*a), 117.2 (C-5′), 117.5 (C-5*′), 117.5 (C-2′), 118.2 (C-2*′), 121.1 (C-6′), 123.5 (C-6*′), 127.4 (C-1*′), 130.6 (C-1′), 138.1 (C-3*), 142.1 (C-3*′), 143.3 (C-4*′), 146.3 (C-2*), 147.6 (C-3′), 147.9 (C-4′), 158.2 (C-8*a), 161.0 (C-8a), 162.5 (C-5*), 165.4 (C-5), 165.8 (C-7*), 169.8 (C-7), 177.4 (C-4*), 189.7 (C-4).

*1,3,11a-Trihydroxy-9-(3,5,7-trihydroxy-4H-1-benzopyran-4-on-2-yl)-5a-(3,4-dihydroxyphenyl)-5,6,11-hexahydo-5,6,11-trioxanaphthacene-12-one (**8**).* Yellow amorphous solid; UV (methanol) λ_{max} (log ϵ) 269 (4.34), 299 (4.37), and 359 nm (4.32); HR-FABMS *m/z* 603.0792 ([M + H]$^+$), calcd for $C_{30}H_{19}O_{14}$, 603.0775; ESIMS (positive) *m/z* 603.10 ([M + H]$^+$, 100%); ESIMS (negative) *m/z* 601.10 ([M − H]$^-$, 10%) and 299.05 ([$C_{15}H_7O_7$]$^-$, 100%); APCIMS (negative) *m/z* 601.10 ([M − H]$^-$, 5%) and 301.00 ([$C_{15}H_9O_7$]$^-$, 100%); ^{1}H NMR (CD_3OD) δ 5.97 (m, 2H, H-6, H-8), 6.179 (d, J = 2.2 Hz, 2/3H, H-6*), 6.184 (d, J = 2.2 Hz, 1/3H, H-6*), 6.38 (brs, 2/3H, H-8*), 6.40 (brs, 1/3H, H-8*), 6.72 (d, J = 8.4 Hz, 2/3H, H-5′), 6.73 (d, J = 8.4 Hz, 1/3H, H-5′), 7.09 (m, 1H, H-6′), 7.17 (d, J = 8.4 Hz, 1H, H-5*′), 7.27 (d, J = 2.2 Hz, 2/3H, H-2′), 7.28 (d, J = 2.2 Hz, 1/3H, H-2′), 7.88 (m, 1H, H-6*′), and 7.97 (d, J = 2.2 Hz, 1H, H-2*′); ^{13}C NMR (CD_3OD) δ 91.9 and 92.1 (C-3), 94.6 (C-8*), 97.4 (C-8), 98.0 (C-6), 99.4 (C-6*), 101.3 (C-4a), 101.9 and 102.0 (C-2), 104.6 (C-4a*), 115.5 (C-5′), 116.8 (C-2′), 117.7 and 117.9 (C-5*′), 118.2 and 118.5 (C-2*′), 121.3 (C-6′), 123.4 and 123.6 (C-6*′), 126.8 and 127.1 (C-1′), 127.3 (C-1*′), 138.1 (C-3*), 141.8 and 142.2 (C-3*′), 143.5 and 143.9 (C-4*′), 145.8 (C-3′), 146.3 (C-2*), 148.0 (C-4′), 158.2 (C-8a*), 161.2 (C-8a), 162.5 (C-5*), 165.4 (C-5), 165.8 (C-7*), 169.7 (C-7), 177.4 (C-4*), and 189.9 (C-4).

*Compound **9**.* Yellow amorphous solid; UV (methanol) λ_{max} (log ϵ) 371 (3.45) and 301 (3.73); ESIMS (positive) *m/z* 903.05 ([M + H]$^+$, 100%); ESIMS (negative) *m/z* 901.05 ([M − H]$^-$, 80%) and 298.90 ([$C_{15}H_7O_7$]$^-$, 100%); ^{1}H NMR (CD_3OD) δ 5.95 (s, 2H, H-6, H-8), 6.02 (s, 2H, H-6**, H-8**), 6.19 (s, 1H, H-6*), 6.40 (brs, 2/ 3H, H-8*), 6.42 (brs, 1/3H, H-8*), 6.68 (d, J = 8.6 Hz, 1/3H, H-5**′), 6.69 (d, J = 8.6 Hz, 2/3H, H-5**′), 6.938 (d, J = 8.6 Hz, 1/3H, H-5′), 6.945 (d, J = 8.6 Hz, 1/2H, H-5′), 6.954 (d, J = 8.6 Hz, 1/6H, H-5′), 7.04 (d, J = 8.6 Hz, 3/5H, H-6**′), 7.05 (d, J = 8.6 Hz, 2/5H, H-6**′), 7.11 (d, J = 8.6 Hz, 1/3H, H-5*′), 7.20 (d, J = 8.6 Hz, 2/ 3H, H-5*′), 7.23 (s, 1H, H-2**′), 7.37 (m, 1H, H-6′), 7.488 (d, J = 8.6 Hz, 1/3H, H-2′), 7.492 (d, J = 8.6 Hz, 1/2H, H-2′), 7.499 (d, J = 8.6 Hz, 1/6H, H-2′), 7.89 (brs, 1H, H-6*′), 7.90 (s, 1/2H, H-2*′), 7.98 (s, 1/6H, H-2*′), 8.00 (s, 1/3H, H-2*′); ^{13}C NMR (CD_3OD) δ 91.8 (C-3), 91.9 (C-3**), 94.6 (C-8*), 97.5 (C-6, C-8, C-6**, C-8**), 99.5 (C-6*), 101.2 (C-4a**), 101.3 (C-2), 101.5 and 101.6 (C-4a), 101.8 (C-2**), 104.6 (C-4*a), 115.5 (C-5**′), 116.8 (C-5*′, C-2**′), 117.9 and 118.0 (C-5′), 118.3 (C-2*′), 118.6 and 118.7 (C-2′), 121.3 (C-6**′), 123.5 (C-6*′), 123.67 and 123.86 (C-6′), 123.9 (C-1′), 126.8 (C-1**′), 127.3 and 127.6 (C-1*′), 138.2 (C-3*), 141.4 and 141.5 (C-3′), 141.7 and 142.1 (C-4*′), 143.3 (C-4′), 143.8 (C-3*′, C-4′), 145.8 (C-3**′), 146.3 (C-2*), 148.0 (C-4**′), 158.3 (C-8*a), 160.9 (C-8**a), 161.2 (C-8a), 162.5 (C-5*), 165.3 (C-5**), 165.5 (C-5), 165.9 (C-7*), 169.8 (C-7), 170.1 (C-7**), 177.5 (C-4*), 189.4 and 189.6 (C-4**), and 189.7 (C-4). Undecamethyl derivative of **9:** APCIMS (positive) *m/z* 1075.45 ([M + H]$^+$, 25%); ^{1}H NMR ($CDCl_3$) δ 3.73 (s, 3H, OC*H*$_3$), 3.81 (s, 3H, OC*H*$_3$), 3.82 (s, 3H, OC*H*$_3$), 3.83 (s, 3H, OC*H*$_3$), 3.85 (s, 3H, OC*H*$_3$), 3.87 (s, 3H, OC*H*$_3$), 3.88 (s, 3H, OC*H*$_3$), 3.90 (s, 3H, OC*H*$_3$), 3.91 (s, 3H, OC*H*$_3$), 3.92 (s, 3H, OC*H*$_3$), 3.95 (s, 3H, OC*H*$_3$), 6.13 (s, 2H, H-6, H-8), 6.16 (s, 2H, H-6**, H-8**), 6.33 (s, 1H, H-6*), 6.49 (s, 1H, H-8*), 6.76 (d, J = 8.6 Hz, 1H, H-5**′), 7.00 (d, J = 8.6 Hz, 1/2H, H-5′), 7.01 (d, J = 8.6 Hz, 1/2H, H-5′), 7.14 (m, 1H, H-5*′), 7.24 (d, J = 9.2 Hz, 1H, H-6**′), 7.31 (s, 1H, H-2**′), 7.36 (m, 1/2H, H-6′), 7.40 (m, 1/2H, H-6′), 7.50 (s, 1/2H, H-2′), 7.52 (s, 1/2H, H-2′), 7.84 (s, 2H, H-2*′, H-6*′).

Antioxidative Activity. The antioxidative activity of each isolated compound was measured by its inhibition against methyl linoleate autoxidation in bulk phase (*19*). Methyl linoleate (294 mg, 1.0 mmol) containing the isolated compound (each 0.1 μmol; 0.01 mol %, based on methyl linoleate) or α-tocopherol (0.01 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 a 36-h incubation, each sample (25 μL) was withdrawn and dissolved in 1.0 mL of ethanol. The peroxide value in

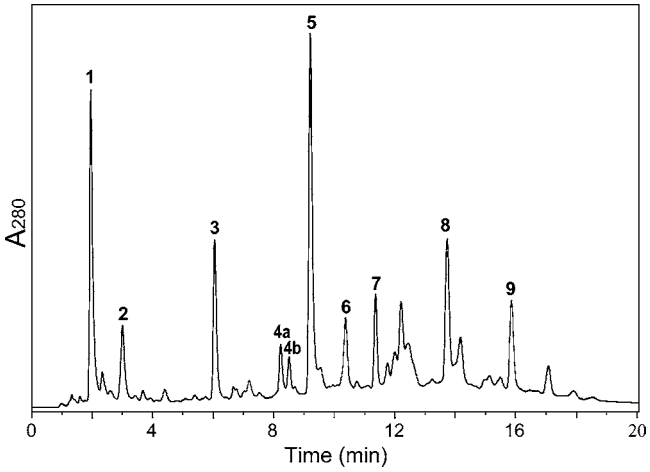

Figure 1. HPLC chromatogram of the methanol fraction from dry outer scales of onion.

each sample solution was determined by the iodometric method (*16*). 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 by the extrusion method (*20*). Soybean PC containing the isolated compound or α-tocopherol (0.1 mol %, based on PC) was suspended by vigorously 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 was 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, 4 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 preparing liposomes. In this case, the liposomes were quickly prepared at 4 °C in order 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 reserved-phase HPLC (*20*).

RESULTS AND DISCUSSION

Structures of Isolated Compounds. Methanol extract from the outer scales of onion was analyzed by reverse-phase HPLC (**Figure 1**). Many peaks including **1**−**9** appeared on the chromatogram detected at 280 nm. For the isolation and characterization of the detected peaks, the methanol extract 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**−**9**, which corresponded to peaks **1**−**9** on **Figure 1**, respectively. The structures of compounds **1**−**9** were characterized as follows (**Figure 2**).

Compound **1** was obtained as a brown amorphous solid (102.7 mg) and identified to be 3,4-dihydroxybenzoic acid (protocatechuic acid) by comparison of its ^{1}H and ^{13}C NMR data with those in the literature values (*21*, *22*). Compound **1** was formed by degradation of quercetin during the autoxidation on browning of onion bulbs (*10*). Protocatechuic acid is well known to be one of the major strong antioxidants from vegetables and fruits, which has been isolated from *Amomum tsao-ko* fruit (*21*), almond skin (*22*), and prunes (*23*). This compound was reported to have potent chemopreventive effects on colon and oral carcinogenesis in rats (*24*) and inhibitory effect on tumor promotion in mouse skin (*25*).

Compound **2** was obtained as a brown-red amorphous solid (18.5 mg). The NMR data of **2** was in agreement with the published values (*26*, *27*), in which **2** was the oxidized product of quercetin, the pyranone C-ring of quercetin being converted to a furanone-carbonyl derivative. Thus, **2** was identified as 2-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxy-3(2*H*)-benzofuranone. Compound **2** was isolated for the first time from dry outer scales of onion.

Compounds **3**, **5**, **7**, and **8** were obtained as yellow or brown-red amorphous solids (**3**, 83.9 mg; **5**, 314.6 mg; **7**, 52.6 mg; **8**, 86.8 mg) and identified as quercetin 4′-*O*-β-D-glucopyranoside (**3**), quercetin (**5**), 1,3,11a-trihydroxy-9-(3,5,7-trihydroxy-4*H*-1-benzopyran-4-on-2-yl)-5a-[4-(β-D-glucopyranosyloxy)-3-hydroxyphenyl]-5,6,11-hexahydro-5,6,11-trioxanaphthacene-12-one (4′-*O*-β-D-glucopyranoside of quercetin dimer, **7**), and 1,3,11a-trihydroxy-9-(3,5,7-trihydroxy-4*H*-1-benzopyran-4-on-2-yl)-5a-(3,4-dihydroxyphenyl)-5,6,11-hexahydo-5,6,11-trioxanaphthacene-12-one (quercetin dimer, **8**), respectively, by comparison of their ^{1}H and ^{13}C NMR data with those of literature values (*9*, *11*, *12*, *27*, *28*). Quercetin 4′-*O*-β-D-glucopyranoside (**3**) is one of the major flavonoids in onion (*7*−*9*). Quercetin (**5**) was formed by hydrolysis of quercetin glucosides (*7*, *10*). The doubly linked oxidative dimer of quercetin (**8**) has been observed during the autoxidation of methyl linoleate solutions containing quercetin (*28*) or during the oxidation of quercetin with a peroxyl radical generator, 2,2′-azobis-isobutyronitrile (*27*). Similarly, compound **7** might be produced by the reaction of quercetin (**5**) with its 4′-glucoside (**3**). Compounds **3**, **5**, **7**, and **8** have already been isolated from the brownish scales of onions (*11*, *12*).

Compounds **4a** and **4b** were obtained as brown-red amorphous solids (15.7 mg and 23.3 mg). HR-FABMS of **4a** and **4b** gave the same molecular formula $C_{22}H_{14}O_{11}$. Their ^{1}H and ^{13}C NMR spectra were very similar to one another. However, differences in chemical shift and coupling constant parameters of the protocatechuic acid moiety were observed. The ^{1}H NMR spectrum of each compound indicates the presence of quercetin moiety (**4a** and **4b**, δ 5.94, 6.69, 7.05, 7.23); trisubstituted benzene protons of protocatechuic acid moiety [**4a**, δ 7.11 (d, J = 8.6 Hz, 1H, H-5*′), 7.60 (d, J = 1.7 Hz, 1H, H-2*′), and 7.70 (d, J = 2.3, 8.6 Hz, 1H, 1H, H-6*′); **4b**, δ 7.02 (d, J = 8.6 Hz, 1H, H-5*′), 7.68 (d, J = 8.6 Hz, 1H, H-6*′), and 7.69 (s, 1H, H-2*′)]. The ^{13}C NMR spectrum of **4a** and **4b** showed 22 carbon signals including characteristic signals of two quaternary carbons (**4a**, δ 91.8 and 102.0; **4b**, δ 92.1 and 101.8), and a carbonyl carbon (**4a**, δ 189.8; **4b**, δ 189.6). Methylation of **4a** and **4b** with diazomethane afforded hexamethyl derivatives, indicating the presence of five hydroxyl groups and one carboxyl group in each molecule. These data indicate that **4a** and **4b** are isomers consisting of quercetin and protocatechuic acid moieties, in which oxidative coupling of the conjugated olefinic linkage (C-2 and C-3) of the C-ring of quercetin and the *o*-dihydroxy group (C-3*′ and C-4*′) of protocatechuic acid gives rise to two regioisomers with either a C2−C4*′/C3−C3*′ or a C2−C3*′/C3−C4*′ dioxane linkage (**Figure 2**). However, the precise structures of **4a** and **4b** could not be determined.

Compound **6** was obtained as a brown-red amorphous solid (45.7 mg). The molecular formula of **6** was the same as **7**, determined by HR-FABMS to be $C_{36}H_{28}O_{19}$. Glucose was detected as the sugar component. The ^{1}H NMR spectrum indicated the presence of two meta-coupled proton signals at δ 5.98 (H-6*) and 6.03 (H-8*), one meta proton signal at 6.42

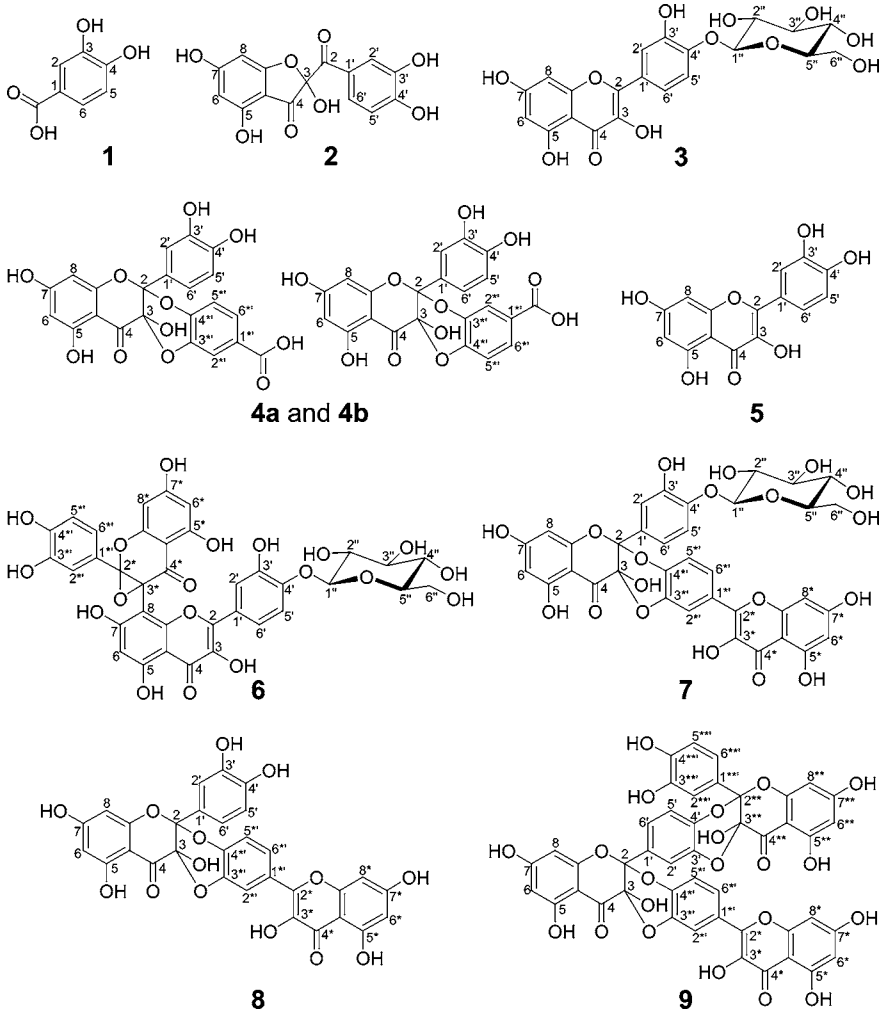

Figure 2. Structures of compounds **1**–**9** isolated from outer scales of onion. The possible structures of **4**, **6**, and **9** are shown.

(H-6), two sets of trisubstituted benzene ring [δ 6.25 and 6.33 (H-6′), 7.00 and 7.04 (H-5′), and 7.76 and 7.78 (H-2′) and 6.816 and 6.820 (H-5*′), 6.86 and 6.87 (H-6*′), and 6.97 and 6.99 (H-2*′)]. The anomeric proton signal at δ 4.85 (d, J = 7.4 Hz, 1/3H, H-1″) or 4.87 (d, J = 7.4 Hz, 2/3H, H-1″) indicates β-configuration of the glucosidic linkage. The ^{13}C NMR of compound **6** showed 36 carbon signals, including anomeric carbon signals at δ 103.2 and 103.7 (C-1″), two quaternary carbon signals at δ 93.67 and 93.71 (C-2*) and 104.4 (C-3*), and two carbonyl carbon signals at δ 177.7 (C-4) and 186.9 (C-4*). These data indicate that the aglycone contains two quercetin molecules, in which the conjugated olefinic linkage (C-2*, C-3*) of the C-ring of one quercetin is substituted to saturated structure at C-2* and C-3* to form a C2*−C3* epoxide, and the carbon at C3* would bind with the carbon at C-6 or C-8 of the A-ring of the other. The structural assignment of **6** was supported by HMBC cross peaks: the anomeric proton signals at δ 4.85 and 4.87 (H-1″) gave strong cross peaks with carbon signals at δ 148.2 and 148.4 (C-4′), these carbon signals then gave cross peaks with proton signals at δ 6.25 and 6.33 (H-6′), 7.76 and 7.78 (H-2′), and these proton signals then gave cross peaks with carbon signals at δ 146.93 and 146.98 (C-2); the proton signals at δ 6.86 and 6.87 (H-6*′) and 6.97 and 6.99 (H-2*′) gave cross-peaks with a carbon signal at δ 93.67 and 93.71 (C-2*); a proton signal at δ 6.42 (H-6) gave cross peaks with the carbon signals at δ 106.3 (C-4a), 165.7 (C-5), 167.6 (C-7), and 108.7 and 108.8 (C-8). These results indicate that the C-1 position of glucose is linked to the hydroxyl group of the C-4′ position of the aglycone and the C-3* position of the C-ring one quercetin might be linked to the C-8 position of the other A-ring. Furthermore, compound **6** was methylated with diazomethane followed by hydrolysis and acetylation to yield an octamethyl ether derivative of the 4′-*O*-acetylaglycone. This indicates that the aglycone has nine hydroxyl groups in the molecule. From these spectral data, one possible structure of compound **6** was assumed to be stereoisomers of 2-[4-(β-D-glucopyranosyloxy)-3-hydroxyphenyl]-3,5,7-trihydroxy-9-[2-(3,4-dihydroxyphenyl)-2,3-dihydro-2,3-epoxy-5,7-dihydroxy-4*H*-1-benzopyrane-4-on-8-yl]-4 *H*-1-benzopyrane-4-one. However, the precise structures could not be determined.

Compound **9** was obtained as a yellow amorphous solid (32.7 mg). The molecular formula of **9** was assumed to be $C_{45}H_{26}O_{21}$ from the low-resolution MS data, although the HR-FABMS could not be determined due to the absence of the molecular ion. Methylation of **9** afforded an undecamethyl ether derivative, indicating the presence of eleven hydroxyl groups in the molecule. The ^{1}H NMR spectrum indicated the presence of three sets of meta-coupled proton signals at δ 5.95 (H-6, H-8), at δ 6.02 (H-6**, H-8**), and at δ 6.19 (H-6*), 6.40 and 6.42 (H-8*); three sets of trisubstituted benzene rings at δ 6.68 and 6.69 (H-5**′), 7.04 and 7.05 (H-6**′), and 7.23 (H-2**′), at δ 6.938, 6.945, and 6.954 (H-5′), 7.37 (H-6′), and 7.488, 7.492 and 7.499 (H-2′), and at δ 7.11 and 7.20 (H-5*′), 7.89 (H-6*′), and 7.90, 7.98, and 8.00 (H-2*′). The ^{13}C NMR of **9** showed 45 carbon signals, including three carbonyl carbon signals at δ 177.5 (C-4*), 189.4 and 189.6 (C-4**), and 189.7 (C-4). The carbon

Table 1. Contents of Compounds **1**–**9** in Onion Outer Scales

compound	amount (mg/g of dry scales)[a]
1	5.02 ± 0.24
2	0.93 ± 0.17
3	3.44 ± 0.20
4a	1.34 ± 0.15
4b	1.07 ± 0.12
5	9.39 ± 0.44
6	2.27 ± 0.20
7	2.21 ± 0.15
8	4.44 ± 0.33
9	2.83 ± 0.25

[a] The amount of each compound was determined by the HPLC analysis as described in Materials and Methods. Each value is expressed as mean ± standard deviation ($n = 3$).

signals at C-2/C-2** and C-3/C-3** were shifted 44.5−46.4 ppm upfield against those at C-2* and C-3*; whereas the carbon signals at C-3**′ and C-4**′ were 2.0−6.3 ppm downfield against those at C-3′/ C-3*′ and C-4′/C-4*′, respectively. These data indicate that **9** is the trimer of quercetin, in which the conjugated olefinic linkage (C-2, C-3) of the C-ring of one quercetin is linked to the *o*-dihydroxyl group (C-3*′, C-4*′) of the second quercetin and the conjugated olefinic linkage (C-2**, C-3**) of the C-ring of the third quercetin is linked to the *o*-dihydroxyl group (C-3′, C-4′) of the first quercetin. The structural assignment of **9** was supported by HMBC correlations (data not shown). Although the two dioxane linkages in the molecule could not be assigned from the present data, the possible structure of compound **9** was assumed to be steroisomers of 1,3,11a-trihydroxy-9-(3,5,7-trihydroxy-4*H*-1-benzopyran-4-on-2-yl)-5a-[1,3,11a-trihydroxy-5a-(3,4-dihydroxyphenyl)-5,6,11-hexahydro-5,6,11-trioxanaphthacene-12-on-9-yl]-5,6,11-hexahydro-5,6,11-trioxanaphthacene-12-one by comparison of the ^{1}H and ^{13}C NMR data with those in the literature values of quercetin dimer (*27*, *28*).

Compounds **1**−**9** in dry outer scales of onion were quantified by reversed-phase HPLC (**Table 1**). The most abundant compound in the outer scales was quercetin (**5**). In addition, protocatechuic acid (**1**), quercetin 4′-*O*-*β*-D-glucopyranoside (**3**), and the doubly linked oxidative dimmer and trimer of quercetin (**8** and **9**) were present in relatively high amounts.

Antioxidative Activities of Compounds 1−**9.** Compounds **1**−**9** are expected to act as antioxidants because these have phenolic hydroxyl groups in the molecules. **Figure 3** shows the inhibitory effect of compounds **1**−**9** 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. Quercetin (**5**) and its dimerized compound (**6**) showed the highest antioxidative activity, which was comparable to that of α-tocopherol. Compounds **5** and **8** had higher antioxidative activity compared with those of the corresponding glucosides (**3** and **7**). Although the positions of phenolic hydroxyl groups of **2** and **5** were the same, **5** showed higher antioxidative activity. These results indicate that the antioxidative activity of the isolated compounds might be influenced by the presence of an *o*-dihydroxy substituent in the B-ring, an olefinic linkage in conjunction with a 4-oxo group in the C-ring, and 3- and 5-hydroxy groups in the A-ring (*29*).

The antioxidative activity of compounds **1**−**9** during the peroxidation of soybean PC liposomes induced by AAPH or AMVN were also evaluated (**Figure 4**). **Figure 4A** shows the effect of compounds **1**−**9** on the AAPH-induced PC peroxidation. The formation of PC−OOH was inhibited by the addition of compounds **1**−**9** or α-tocopherol. Compounds **4**, **5**, **8**, and **9** especially showed the highest antioxidative activity. 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 **4**, **5**, **8**, and **9** 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, the effect of compounds **1**−**9** or α-tocopherol was

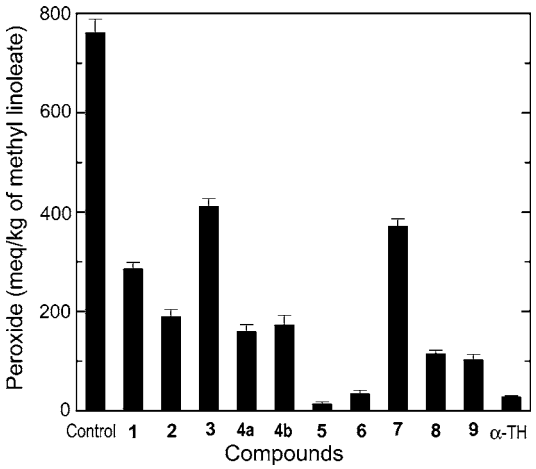

Figure 3. Effect of compounds **1**–**9** on the autoxidation of methyl linoleate in bulk phase. Each value is expressed as mean ± standard deviation of three different experiments.

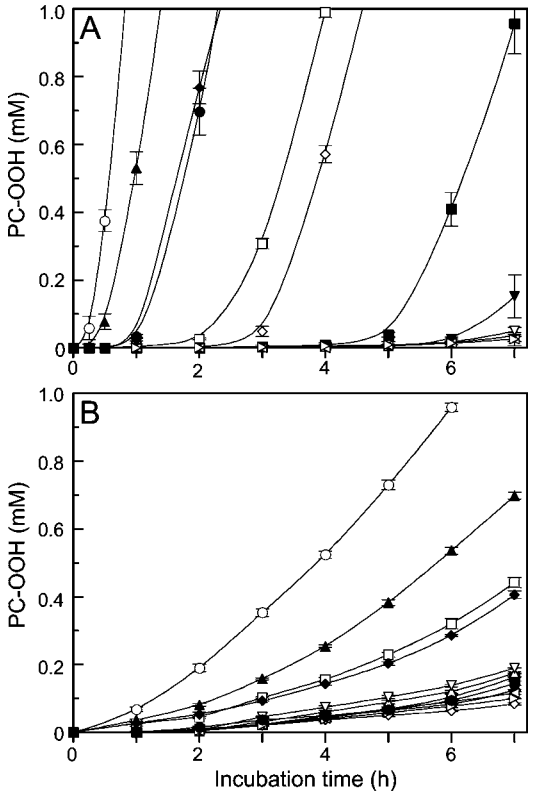

Figure 4. Effect of compounds **1**–**9** on the AAPH-induced (A) and AMVN-induced (B) peroxidation of soybean PC in liposomes. ○, No addition; □, **1**; ■, **2**; ▲, **3**; △, **4a**; ▽, **4b**; ▼, **5**; ◇, **6**; ◆, **7**; triangle pointing left, **8**; triangle pointing right, **9**; and ●, α-tocopherol. Each value is expressed as mean ± standard deviation of three different experiments.

slightly different (**Figure 4B**). In addition to compounds **4**, **5**, **8**, and **9**, α-tocopherol and compounds **2** and **6** also suppressed the peroxidation effectively. α-Tocopherol is a well-known lipid-soluble antioxidant. Thus, α-tocopherol and compounds **4**−**6**, **8**, and **9** might be located on the surface or inside of the membranes where they scavenge peroxyl radicals generated in the lipid phase. In the peroxidation of PC liposomal systems, it should be recognized that the antioxidant activity depends not only on the structure of antioxidants but also on the affinity with lipid bilayer (*30*).

This study shows that the dry outer scales of onion contain large amounts of quercetin (**5**), quercetin glucoside (**3**), and their oxidative products (**1**, **2**, **4**, **6**−**9**). These compounds could suppress the autoxidation of methyl linoleate in bulk phase and the radical-initiated peroxidation of soybean phosphatidylcholine in liposomes. Therefore, the outer scale extract of onion is expected to be a resource for food ingredients.

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