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# Dihydrochalcones: Evaluation as Novel Radical Scavenging Antioxidants

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Dihydrochalcones are a family of bicyclic flavonoids, defined by the presence of two benzene rings joined by a saturated three carbon bridge. In the present study, we systematically examined the antioxidant activities of dihydrochalcones against the stable free radical (1,1-diphenyl-2-picrylhydrazyl) and lipid peroxidation in the erythrocyte membrane. All dihydrochalcones exhibited higher antioxidant activities than the corresponding flavanones. The $^1$H NMR analysis indicated that the active dihydrochalcone has a time-averaged conformation in which the aromatic A ring is orthogonal to the carbonyl group, while the inactive dihydrochalcone such as 2′-*O*-methyl-phloretin has a strongly hydrogen-bonded phenolic hydroxyl group, suggestive of a coplanar conformation. A hydroxyl group at the 2′-position of the dihydrochalcone A ring, newly formed by reduction of the flavanone C ring, is an essential pharmacophore for its radical scavenging potential.

**KEYWORDS: Antioxidant; dihydrochalcone; 1,1-diphenyl-2-picrylhydrazyl; lipid peroxidation; flavanone; erythrocyte**

## INTRODUCTION

Reactive oxygen species (ROS) and free radicals induce membrane damage, DNA base oxidation, DNA strand breaks, chromosomal aberrations, and protein alterations. Some ROS attack unsaturated fatty acids and cause oxidative damage to the cell membrane. Therefore, oxidative stress is thought to be closely associated with aging, atherosclerosis, and carcinogenesis (*1*). Living cells normally protect themselves from oxidative damage by low molecular weight antioxidants including tocopherols and glutathione as well as antioxidant enzymes. However, it is noted that these defense systems are damaged and disrupted in a pathologically oxidative situation (*2*). Besides the endogenous antioxidants, there is, therefore, increasing interest in the protective and preventive function of foods and their constituents against oxidative stress caused by ROS (*3*).

Flavonoids, including flavones, flavonols, isoflavones, and flavanones, are one type of polyphenol common in vegetables and fruits. These display a remarkable spectrum of biochemical activities such as antioxidant activities, antimutagenesis, cytotoxic activities, and altered gene expression (*4*). Dihydrochalcones are a family of the bicyclic flavonoids, defined by the presence of two benzenoid rings joined by a three carbon bridge. Neohesperidin dihydrochalcone (**5b**), the most familiar of these compounds, is an intense sweetener and permitted for commercial use as a food additive in Europe on the basis of its safety assessed by the EU Scientific Committee for Food (*5*). Some dihydrochalcones including phloridzin and myrigalone B have been found to occur naturally (*6*, *7*) and to possess an antioxidant activity (*8*, *9*). Chemically, dihydrochalcones can be easily prepared by catalytic hydrogenation under alkaline condition from flavanones, most of which, together with glucosides, are common in the genus *Citrus* and specific *Citrus* cultivars (*5*). Although a large number of antioxidants, including most types of flavonoids, have been reported in the literature, there are few systematic studies of dihydrochalcones on biological activities such as an antioxidant effect.

In the present study, to evaluate dihydrochalcones as novel antioxidant materials for pharmaceutical agents or food additives, their free radical scavenging activity and inhibitory effect on lipid peroxidation were determined. In addition, to investigate the structure−activity relationship, the inhibitory effect of the structurally related derivatives including flavanones was also estimated. We thus confirmed that a hydroxyl group at the 2′-position of the dihydrochalcone A ring, newly formed by reduction of the flavanone C ring, is an essential pharmacophore for its radical scavenging potential.

## MATERIALS AND METHODS

**General Procedures.** The analytical instruments used were as follows: FAB-MS, JEOL JMS-700 MStation; NMR, Bruker ARX-400 spectrometer with tetramethylsilane as the internal standard.

**Chemicals.** Narigenin (**1a**), phloretin (**1b**), eriodictyol (**4a**), neohesperidin (**5a**), and **5b** were purchased from Extrasynthése (Genay,

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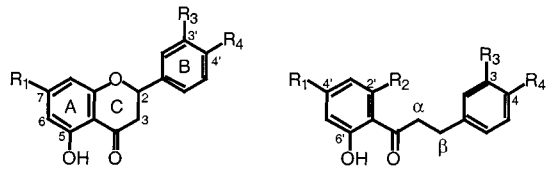


| No. | name | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|---|
| **1a** | naringenin | OH | - | H | OH |
| **1b** | phloretin | OH | OH | H | OH |
| **2a** | isosakuranetin | OH | - | H | $OCH_3$ |
| **2b** | isosakuranetin dihydrochalcone | OH | OH | H | $OCH_3$ |
| **3a** | hesperetin | OH | - | OH | $OCH_3$ |
| **3b** | hesperetin dihydrochalcone | OH | OH | OH | $OCH_3$ |
| **4a** | eriodictyol | OH | - | OH | OH |
| **4b** | eriodictyol dihydrochalcone | OH | OH | OH | OH |
| **5a** | neohesperidin | ONeo | OH | OH | $OCH_3$ |
| **5b** | neohesperidin dihydrochalcone | ONeo | OH | OH | $OCH_3$ |
| **6a** | hesperidin | ORut | OH | OH | $OCH_3$ |
| **6b** | hesperidin dihydrochalcone | ORut | OH | OH | $OCH_3$ |
| **7b** | 2′-*O*-methyl-phloretin | OH | $OCH_3$ | H | OH |
| **8b** | 4′-*O*-methyl-phloretin | $OCH_3$ | OH | H | OH |
| **9a** | naringin | ONeo | - | H | OH |
| **9b** | naringin dihydrochalcone | ONeo | OH | H | OH |
| **10b** | asebotin | OH | OGlu | H | OH |
| **11b** | phloridzin | $OCH_3$ | OGlu | H | OH |

**Figure 1.** Structures of flavanones (group **a**) and dihydrochalcones (group **b**). ONeo, *O*-neohesperidoside; ORut, *O*-rutinoside; OGlu, *O*-glucoside.

France). Isosakuranetin (**2a**) was obtained from Apin Chemicals, Ltd. (Abingdon, U.K.). Hesperetin (**3a**) and phloridzin (**11b**) were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Hesperidin (**6a**) was purchased from Alps Pharmaceutical Industry Co., Ltd. (Gifu, Japan). The structures of flavanones and dihydrochalcones are shown in **Figure 1**. All other chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan).

**Preparation of Dihydrochalcones.** Isosakuranetin dihydrochalcone (**2b**), hesperetin dihydrochalcone (**3b**), eriodictyol dihydrochalcone (**4b**), and hesperidin dihydrochalcone (**6b**) were prepared by hydrogenation of flavanones as previously reported (*10*). Briefly, 5% palladium on carbon (1 g) was added to a flavanone solution (10 mmol) in 10% potassium hydroxide (50 mL). The mixture was shaken with hydrogen (3 kg/cm$^2$) at 30 °C for 30 min. After it was filtered, the filtrate was neutralized with hydrogen chloride and recrystallized. The final purification was done by absorption column chromatography with Diaion HP-20 resin to afford the corresponding dihydrochalcones as crystals in good yields (~80%). The corresponding products were identical to **2b**, **3b**, and **4b** as previously reported (*11*−*13*). Compound **6b**: $^1$H NMR (500 MHz, $CD_3OD$): $\delta$ 1.17 (3H, d, $J$ = 6 Hz, rha-$CH_3$), 2.76 (2H, t, $J$ = 7 Hz, α), 3.24 (2H, t, $J$ = 7 Hz, $\beta$), 3.30−3.96 (10H, d, sugar), 3.78 (3H, s, 4-$OCH_3$), 4.80, 5.04 (2H, d, $J$ = 7 Hz, anomeric proton), 6.10 (2H, s, 3′, 5′-H) 6.66 (1H, dd, $J$ = 2, 8 Hz, 6-H), 6.72 (1H, d, $J$ = 2 Hz, 2-H), 6.84 (1H, d, $J$ = 8 Hz, 5-H). $^{13}$C NMR (75 MHz, $CD_3OD$): $\delta$ 17.4 (Rha-$CH_3$), 30.8 ($\beta$), 46.4 (α), 56.9 ($OCH_3$), 66.8 (Glu-6), 69.6, 70.4, 71.1, 71.3, 73.2 (Glu-4, Rha-2, 3, 4, 5), 73.8, 76.0, 76.6 (Glu-2, 3, 5), 96.6 (3′), 96.6 (5′), 99.9 (Glu-1), 101.3 (Rha-1), 107.1 (1′), 113.6, 116.5 (2), 121.2 (6), 135.7 (1), 144.5 (3), 146.6 (4), 163.8 (4′), 164.1 (2′), 164.1 (6′), 208.1 (C═O). FAB-MS (matrix; glycerol) *m*/*z*: 613 ([M + H]$^+$).

**Preparation of 2′-*O*-Methyl and 4′-*O*-Methyl Derivatives of 1b.** 2′-Methyl-phloretin (**7b**) and 4′-*O*-methyl-phloretin (**8b**) were prepared by methylation and sugar hydrolysis of naringin dihydrochalcone (**9b**) and asebotin (**10b**), respectively. Compound **9b** was prepared by the reduction of narindin (**9a**) according to the procedure described above (*10*). Compound **9b**: $^1$H NMR (500 MHz, $(CD_3)_2SO$): $\delta$ 1.21 (3H, d, $J$ = 6 Hz, Rha-$CH_3$), 2.81 (2H, m, α), 3.24 (2H, t, $J$ = 7 Hz, $\beta$), 3.25−5.30 (sugar proton), 5.13 (2H, d, $J$ = 7 Hz, anomeric proton), 6.04 (2H, s, 3′, 5′-H), 6.69 (1H, d, $J$ = 8 Hz, 3, 5-H), 7.05 (2H, d, $J$ = 8 Hz, 2, 6-H), 9.10 (1H, brs, 4-OH), 12.31 (2H, brs, 2′, 6′-OH). $^{13}$C NMR (75 MHz, $(CD_3)_2SO$): $\delta$ 18.1 (Rha-$CH_3$), 29.3 ($\beta$), 45.7 (α), 60.3 (Glu-6), 68.3, 69.5, 70.4, 70.5, 71.9 (Glu-4, Rha-2, 3, 4, 5), 76.6, 76.8, 77.1 (Glu-2, 3, 5), 94.8 (3′), 94.8 (5′), 97.1 (Rha-1), 100.6 (Glu-1), 105.3 (1′), 115.1 (3), 115.1 (5), 129.1 (2), 129.1 (6), 131.5 (1), 155.4 (4), 162.9 (4′), 163.7 (2′), 163.7 (6′), 205.1 (C═O). FAB-MS (matrix; glycerol) *m*/*z*: 583 ([M + H]$^+$). To a solution of **9b** (1.2 g) in methanol−acetonitrile (1:9, v/v, 50 mL) was added diisopropylamine (0.26 g) and 2.0 M trimethylsilyldiazomethane solution in hexane (3 mL), and the mixture was kept at room temperature for 1 h while stirring. Then, after 20% HCl (30 mL) added, the mixture was stirred in a water bath (100 °C) for 30 min. The mixture was neutralized with sodium hydroxide and then extracted with ethyl acetate. After the ethyl acetate layer was concentrated in vacuo, the final purification was done by absorption column chromatography with Diaion HP-20 resin to afford the product as crystals. This product (236 mg, 41%) was identical to **7b** on the basis of the spectroscopic data as previously reported (*11*). Compound **10b** was also prepared by 4′-position specific methylation of **11b** according to the same procedure using trimethylsilyldiazomethane. Compound **10b** (39%): $^1$H NMR (500 MHz, $(CD_3)_2SO$): $\delta$ 2.80 (2H, m, $\beta$), 3.30−3.70 (8H, α and sugar proton), 3.78 (3H, s, 4′-$OCH_3$), 4.62 (1H, t, $J$ = 5 Hz, Glu-6-OH), 5.01 (1H, d, $J$ = 7 Hz, anomeric proton), 5.05, 5.14, 5.30 (3H, d, $J$ = 5 Hz, Glu-2,3,4-OH), 6.13 (1H, d, $J$ = 2 Hz, 5′-H), 6.29 (2H, d, $J$ = 2 Hz, 3′-H), 6.64 (2H, d, $J$ = 8 Hz, 3, 5-H), 7.03 (2H, d, $J$ = 8 Hz, 2, 6-H), 9.08 (1H, s, 4-OH), 13.34 (1H, s, 6′-OH). $^{13}$C NMR (75 MHz, $(CD_3)_2SO$): $\delta$ 28.8 ($\beta$), 45.1 (α), 55.6 ($OCH_3$), 60.7 (Glu-6), 69.7 (Glu-4), 73.2 (Glu-2), 76.7 (Glu-5), 77.4 (Glu- 3), 93.5 (3′), 95.1 (5′), 100.8 (Glu-1), 106.2 (1′), 115.0 (3), 115.0 (5), 129.1 (2), 129.1 (6), 131.4 (1), 155.3 (4), 160.2 (2′), 164.9 (6′), 165.1 (4′), 205.1 (C═O). FAB-MS (matrix; glycerol) *m*/*z*: 451 ([M + H]$^+$). To a solution of **10b** (300 mg) in ethanol was added 20% HCl (9 mL), and the mixture was stirred in a water bath (100 °C) for 30 min. The mixture was neutralized with sodium hydroxide and then extracted with ethyl acetate. After the ethyl acetate layer was concentrated in vacuo, the final purification was done by absorption column chromatography with Diaion HP-20 resin to afford the product as crystals. This product (163 mg, 84%) was identical to **8b** on the basis of the spectroscopic data as previously reported (*11*).

**Evaluation of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity.** The DPPH radical scavenging activity was evaluated as reported previously (*14*). A test compound (ethanol solution, 2 mL) mixed with a 100 mM Tris-HCl buffer (pH 7.4, 2 mL) was added to 0.5 mM DPPH in ethanol (1 mL), and the mixture was shaken vigorously and kept for 20 min at room temperature in the dark. The DPPH radical scavenging activity is expressed as the ratio of the relative decrease in the absorbance of the test sample mixture at 517 nm to that of the 1 mM Trolox solution. The experiment was done in triplicate. DPPH radical scavenging activity (%) = {(ethanol alone) − (test compound)}/{(ethanol alone) − (Trolox)} × 100.

**Antioxidant Assay of Rabbit Erythrocyte Membrane Ghost System.** Commercially available rabbit blood was obtained from Japan Biotest Institute Co., Ltd. The preparation of erythrocyte membrane ghost and peroxidation induced by *tert*-butylhydroperoxide were performed by the procedure previously reported (*15*). In brief, rabbit blood (100 mL) was diluted with 300 mL of 10 mM phosphate buffer (pH 7.4) containing 152 mM NaCl. After it was centrifuged (3500 rpm, 20 min), the blood was lysed in 300 mL of 10 mM phosphate buffer (pH 7.4) without NaCl. The erythrocyte membrane ghosts were pelleted by centrifugation (11 500 rpm, 40 min), and the precipitate was diluted to give a suspension (1 mg protein/mL). The test compound dissolved in 25 $\mu$L of dimethyl sulfoxide and 25 $\mu$L of 24 mM *tert*-butylhydroperoxide aqueous solution were added to the ghost suspension (450 $\mu$L), and the mixture thus obtained was incubated at 37 °C for 20 min. The quantity of thiobarbituric acid-reacting substances was determined according to the method of Osawa et al. (*16*). The experiment was done in triplicate.

## RESULTS AND DISCUSSION

**Antioxidant Activities of Dihydrochalcones and Flavanones.** Antioxidant assays were performed by a DPPH

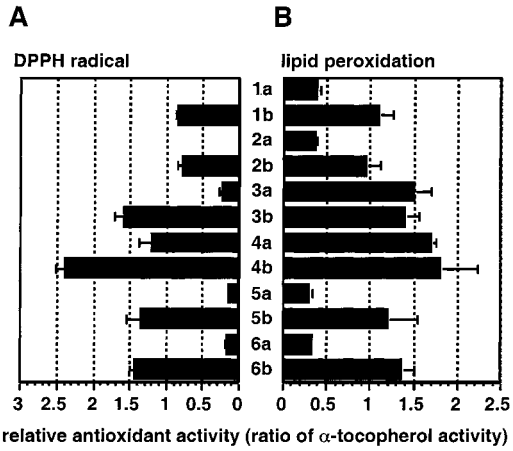


**Figure 2.** Radical scavenging (**A**) and antiperoxidation (**B**) activities of flavanones (**1a**, **2a**, **3a**, **4a**, **5a**, and **6a**) and dihydrochalcones (**1b**, **2b**, **3b**, **4b**, **5b**, and **6b**).

method (*14*), and a rat erythrocyte membrane ghost system (*15*) was used as a model for biological membrane peroxidation as previously reported. Although DPPH is artificial, stable, and thus different from highly reactive oxygen radicals such as hydroxyl, peroxyl, superoxide anion, etc., it has been reported that electrochemical measurements of antioxidant activity or inhibition of lipid peroxidation in the biological membrane shows a positive correlation to DPPH scavenging activity in most cases (*17*−*19*). α-Tocopherol was used as a positive control, and antioxidant activities were evaluated by the concentration required to cause 50% inhibition ($IC_{50}$) as compared to that of α-tocopherol. The $IC_{50}$ values of α-tocopherol were 21 (DPPH) and 134 $\mu$M (lipid peroxidation), respectively.

The antioxidant effects of naturally occurring flavanones (**a**) and the structurally related dihydrochalcones (**b**) are summarized in **Figure 2**. Flavanones, except for **4a**, were determined to be inactive in the DPPH system, of which the activity was much weaker than that of α-tocopherol (**Figure 2A**). Flavanones **1a** and **2a** especially did not show any scavenging activity. Because radical scavenging by phenolic compounds is believed to involve the loss of the phenolic hydrogen atom, it is assumed that a hydroxyl group at the 5- or 7-position of flavanone might not participate in scavenging radicals. On the other hand, the scavenging activity of **4a**, having a catechol moiety in the B ring, was comparative to those of α-tocopherol in both assays. Eriodictyol and its glucosides have recently been identified as potent antioxidants from lemon fruits (*15*). It is indicated that a catechol group in the B ring is an antioxidant pharmcophore for hydrogen atom donation by eriodictyol or its derivatives. In contrast to the DPPH assay, all flavanones exhibited significant antiperoxidation activity in the membrane ghost system (**Figure 2B**). Flavanones **1a** and **2a** expectedly exhibited a weaker antioxidant effect in a lipid peroxidation test than did α-tocopherol. It should be noted that **1a** and **2a**, having no radical scavenging activity, act as antioxidants in the membrane system. Because the lipid peroxidation process is complicated by an initiation step characterized as hydrogen extraction by free radical species and a propagation step by a radical chain reaction, a variety of reactions other than proton donation, for example, stable adduct formation with a free radical, might be involved in the antioxidant action of flavanones (*20*). In addition, metal ion for the production of additional free radicals might be involved in our lipid peroxidation system. Therefore, the chelating action of flavanones cannot be excluded in the antioxidant mechanism (*21*).

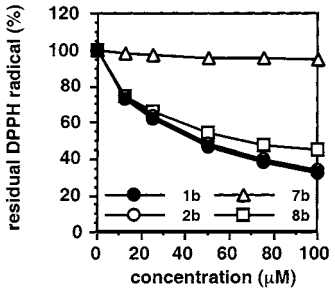


**Figure 3.** DPPH radical scavenging activity of **1b**, **2b**, **7b**, and **8b**. The maximal standard deviation for each experiment was 5% ($n = 3$).

**Table 1.** $^1$H NMR Spetra of **1b** and Its *O*-Methyl Derivatives (**2b**, **7b**, and **8b**)[a]

| substance | 4-OH | 4′-OH | 2′,6′-OH | 3′,5′-H |
|---|---|---|---|---|
| **1b** | 8.08 (brs) | 9.28 (brs) | 11.72 (brs) | 5.88 (s) |
| **2b** | | 9.28 (brs) | 11.72 (brs) | 5.88 (s) |
| **7b** | 8.08 (brs) | 9.40 (brs) | 13.72 (s) | 5.91 (d), 5,98 (d) |
| **8b** | 8.06 (brs) | | 11.78 (brs) | 5.94 (s) |

[a] Chemical shift values are in ppm; brs, broad singlet; s, sharp singlet.

Antioxidant effects of dihydrochalcones (**1b**−**6b**) were significant and much stronger than those of the corresponding flavanones (**1a**−**6a**) in both systems, except that the activities of **3b** and **4b** in the membrane ghost system were as strong as those of **3a** and **4a**, respectively. The activity of each dihydrochalcone was comparable to that of α-tocopherol. Although aglycones are commonly regarded as more potent antioxidants than their corresponding glycosides (*21*), 7-*O*-glycosides, including the sweetening food additive **5b**, also exhibited similar activity to the aglycone **3b**, while the corresponding flavanones **5a** and **3a** were less effective. It is therefore reasonable to assume that a hydroxyl group at the 2′-position of the dihydrochalcone A ring, formed by reduction of the flavanone C ring, is essential for its radical scavenging potential. Previous studies using hydroxylated acetophenone derivatives (*9*, *22*) support this assumption. Moreover, **1b** and **2b**, having no antioxidant pharmacophore in the B ring, showed significant radical scavenging activity despite no scavenging potential of the corresponding flavanones, **1a** and **2a**, also substantiating this idea. In addition, the antioxidant activities of **3b** or **4b** were much stronger than those of **2b** or **1b**, respectively, indicating that substitution by a hydroxyl group at the 3-position of the B ring might cause an enhancing effect.

**Importance of the 2′-Hydroxyl Group of Dihydrochalcones for Radical Scavenging Activity.** To support the above assumption, we prepared and compared the radical scavenging activity of the *O*-methyl derivatives (**2b**, **7b**, and **8b**) with that of **1b**. As shown in **Figure 3**, 4-*O*-methylated **2b** and 4′-*O*-methylated **8b** maintained a scavenging potential comparable to that of **1b**. Thus, participation of hydroxyl groups at the 4- and 4′-position in the hydrogen donation to a free radical could be ruled out. On the other hand, 2′-*O*-methylated **7b** showed little activity in the DPPH system. In addition, 2,4,6-hydroxyacetophenone, having the partial structure of dihydrochalcone, also exhibits significant antioxidant activity comparable with **1b** (data not shown). It is therefore very likely that a 2′-hydroxyl group is involved in radical scavenging activity.

The NMR analysis (**Table 1**) of phloretin derivatives implied that **1b**, **2b**, and **8b** seem to have an orthogonal averaged

conformation on the side chain as suggested by the symmetry of the spectroscopic signals for the 3′ and 5′ protons at 5.88 ppm and the broad signals of the hydrogen-bonded protons. Inactive **7b**, in contrast, showed a unique spectrum characterized by asymmetric signals (3′ proton, 5.91 ppm; 5′ proton, 5.98 ppm) and a sharp hydroxyl signal (13.7 ppm), indicating a coplanar conformation due to a strong hydrogen bond with the carbonyl group. Therefore, the proton of the hydroxyl group at the 6′-position of **7b** is unlikely to be available for proton donation. The observation of the stable asymmetrical conformation of **7b** led us to the hypothesis, first proposed by Mathiesen et al. (*9*), that an active dihydrochalcone after acting as a radical scavenger may form a phenoxy radical, followed by loss of its symmetrical structure. It may then change into a coplanar conformation due to an intramolecular hydrogen bond between the remaining hydroxyl group and the carbonyl group. To test the hypothesis that the scavenging activity of **1b** is driven by formation of an intramolecular hydrogen bond, $^1$H NMR analysis of **1b** upon incubation with DPPH radical was performed without purification (data not shown). When **1b** was treated with DPPH, the sharpened spectra assigned to hydroxyl groups on the A ring (10.3 and 12.2 ppm) were observed. Therefore, **1b** might lose its symmetrical structure.

Other possible mechanisms were considered. Rezk et al. (*22*) have proposed that the stabilization of the radical that is formed after hydrogen abstraction may take part in the antioxidant activity of **1b**. This may involve a keto−enol tautomeric transformation between the carbonyl group and the α-methylene. This explanation is confirmed by the strong activity of 2,6-hydroxyacetophenone. In any case, it should be noted that the 2,4,6-hydroxyacetophenone moiety is a unique pharmacophore responsible for the antioxidant activity of dihydrochalcones, which is quite different from the case of other flavonoids.

In summary, the results in the present study provide the evidence that dihydrochalcones have a significant ability to show radical scavenging activity and thus suppress lipid peroxidation. The stability of food ingredients under normal conditions of use and storage is a critical determinant of the suitability for a particular application. From this aspect, international acceptance of some dihydrochalcones with their stability and safety was manifested by several favorable assessments (*5*). Dihydrochalcones have some advantages for application as food additives because of their safety, stability, colorless property, and yet easy preparation by the usual hydrogenation of citrus flavanones. Further investigation of dihydrochalcones is necessary and will contribute to antioxidaive applications to humans.

## LITERATURE CITED


(1) Olinski, R.; Gackowski, D.; Foksinski, M.; Rozalski, R.; Roszkowski, K.; Jaruga, P. Oxidative DNA damage: Assessment of the role in carcinogenesis, atherosclerosis, and acquired immunodeficiency syndrome. *Free Radical Biol. Med.* **2002**, *33*, 192−200.

(2) Fujii, J.; Taniguchi, N. Down regulation of superoxide dismutases and glutathione peroxidase by reactive oxygen and nitrogen species. *Free Radical Res.* **1999**, *31*, 301−308.

(3) Kris-Etherton, P. M.; Hecker, K. D.; Bonanome, A.; Coval, S. M.; Binkoski, A. E.; Hilpert, K. F.; Griel, A. E.; Etherton, T. D. Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. *Am. J. Med.* **2002**, *113* (Suppl. 9B), 71S−88S.

(4) Tapiero, H.; Tew, K. D.; Ba, G. N.; Mathe, G. Polyphenols: Do they play a role in the prevention of human pathologies? *Biomed. Pharmacother.* **2002**, *56*, 200−207.

(5) Borrego, F.; Canales, I.; Lindley, M. G. Neohesperidin dihydrochalcone: state of knowledge review. *Z. Lebensm. Unters. Forsch.* **1995**, *200*, 32−37.

(6) El-Naggar, S. F.; El-Feraly, F. S.; Foos, J. S.; Doskotch, R. W. Flavonoids from the leaves of *Kalmia latifolia*. *J. Nat. Prod.* **1980**, *43*, 739−751.

(7) Malterud, K. E. C-methylated dihydrochalcones from *Myrica gale* fruit exudate. *Acta Pharm. Nord.* **1992**, 65−68.

(8) Dziedzic, S. Z.; Hudson, B. J. F.; Barnes, G. Polyhydroxydihydrochalcones as antioxidants for lard. *J. Agric. Food Chem.* **1985**, *33*, 244−246.

(9) Mathiesen, L.; Malterud, K. E.; Sund, R. B. Hydrogen bond formation as basis for radical scavenging activity: A structure−activity study of C-methylated dihydrochalcones from *Myrica gale* and structurally related acetophenones. *Free Radical Biol. Med.* **1997**, *22*, 307−311.

(10) Esaki, S.; Nishiyama, K.; Sugiyama, N.; Nakajima, R.; Takao, Y.; Kamiya, D. Preparation and taste of certain glycosides of flavones and of dihydrochalcones. *Biosci., Biotechnol., Biochem.* **1994**, *58*, 1479−1485.

(11) Mizuno, M.; Kojima, H.; Tanaka, T.; Iinuma, M.; Kimira, R.; Zhi-da, M.; Murata, H. Phenolic constituents from seeds of *Coptis japonica* var. *dissecta*. *Phytochemistry* **1987**, *26*, 2071−2074.

(12) Ito, K.; Itoigawa, M.; Haruna, M.; Murata, H.; Furukawa, H. Dihydrochalcones from *Balanophora tobiracola*. *Phytochemistry* **1980**, *19*, 476−477.

(13) Krishnamurty, H. G.; Sathyanarayana, S. Catalytic transfer hydrogenation, a facile conversion of hydroxyflavanones into hydroxydihydrochalcones. *Synth. Commun.* **1989**, *19*, 119−123.

(14) Yamaguchi, T.; Takamura, H.; Matoba, T.; Terao, J. HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. *Biosci., Biotechnol., Biochem.* **1998**, *62*, 1201−1204.

(15) Miyake, Y.; Yamamoto, K.; Osawa, T. Isolation of eriocitrin (eriodictyol 7-rutinoside) from lemon fruit (*Citrus limon* Burm. f.) and its antioxidative activity. *Food Sci. Technol. Int. Tokyo* **1997**, *3*, 84−89.

(16) Osawa, T.; Ide, A.; Su, J.-D.; Namiki, M. Inhibition of lipid peroxidation by ellagic acid. *J. Agric. Food Chem.* **1987**, *35*, 808−812.

(17) Malterud, K. E.; Farbrot, T. L.; Huse, A. E.; Sund, R. B. Antioxidant and radical scavenging effects of anthraquinones and anthrones. *Pharmacology* **1993**, *47* (Suppl. 1), 77−85.

(18) Cos, P.; Rajan, P.; Vedernikova, I.; Calomme, M.; Pieters, L.; Vlietinck, A. J.; Augustyns, K.; Haemers, A.; Vanden Berghe, D. In vitro antioxidant profile of phenolic acid derivatives. *Free Radical Res.* **2002**, *36*, 711−716.

(19) Kahkonen, M. P.; Heinonen, M. Antioxidant activity of anthocyanins and their aglycons. *J. Agric. Food Chem.* **2003**, *51*, 628−633.

(20) Sun, M.; Yamauchi, R.; Ashida, H.; Kanazawa, K. Subsequent products after antioxidant actions of β-carotene and α-tocopherol have no Salmonella mutagenicity. *Biosci., Biotechnol., Biochem.* **2002**, *66*, 363−372.

(21) Heim, K. E.; Tagliaferro, A. R.; Bobilya, D. J. Flavonoid antioxidants: Chemistry, metabolism and structure−activity relationships. *J. Nutr. Biochem.* **2002**, *13*, 572−584.

(22) Rezk, B. M.; Haenen, G. R.; van der Vijgh, W. J.; Bast, A. The antioxidant activity of phloretin: The disclosure of a new antioxidant pharmacophore in flavonoids. *Biochem. Biophys. Res. Commun.* **2002**, *295*, 9−13.


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