

# **DPPH Radical Scavenging Reaction of Hydroxy- and Methoxychalcones**

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The DPPH radical scavenging activity of 2',4',6'trihydroxy- and 2'-hydroxy-4',6'-dimethoxychalcones carrying a 2,3- and 3,4-dihydroxylated, and 3,4,5trihydroxylated B-ring was evaluated in alcoholic and non-alcoholic solvents. All test compounds scavenged more than two equivalent of radicals by a possible conversion to the corresponding B-ring quinones and in most cases subsequently underwent cyclization to aurones and flavanones, these being identified in the reaction solutions by an *in situ* NMR analysis. Interestingly, the reaction between 2',3,4-trihydroxy-4',6'-dimethoxychalcone and the DPPH radical was significantly affected by the solvent used, which might be accounted for by the difference in readiness for cyclization to an aurone.

Key words: chalcone; aurone; flavanone; radical scavenging reaction; DPPH radical

The implication of oxidative and free radical mediated reactions in degenerative processes related to aging and other disease conditions is cause for concern. Free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS) are implicated in numerous pathological conditions such as inflammation, metabolic disorders, cellular aging, reperfusion damage, atherosclerosis, and carcinogenesis.<sup>1,2)</sup>

Polyphenol compounds such as protocatechuic acid, caffeic acid and a variety of flavonoids are present in fruits and vegetables and are an integral part of the human diet. It is already known that dietary polyphenols show potent antiradical ability. The radical scavenging abilities of these phenolic compounds depend greatly on the number and arrangement of phenolic hydroxyl groups. The antiradical reaction of protocatechuic acid,<sup>3–5)</sup> gallic acid,<sup>5)</sup> caffeic acid,<sup>6,7)</sup> quercetin<sup>8)</sup> and catechin<sup>9)</sup> have been widely investigated. It has been that the radical scavenging reactivity of protocatechuic acid and gallic acid changed in protic and aprotic solvents.<sup>3–5)</sup>

Although hydroxychalcones are key compounds precedent to many flavonoids and can be easily synthesized by condensing the corresponding acetophenone with a benzaldehyde, there has been little investigation on their antiradical mechanism and structureactivity relationship.

In this study, to examine the effects of the number and arrangement of hydroxyl groups in the chalcone skeleton on the antiradical activity, 2',3,4-trihydroxy-4',6'-dimethoxychalcone (1), 2',3,4,4',6'-pentahydroxychalcone (2), 2,2',3-trihydroxy-4',6'-dimethoxychalcone (3), 2,2',3,4',6'-pentahydroxychalcone (4), 2',3,4,5-tetrahydroxy-4',6'-dimethoxychalcone (5), and 2',3,4,4',5,6'-hexahydroxychalcone (6) were prepared and tested for their DPPH radical scavenging activity in aprotic and protic solvents.

## **Materials and Methods**

*Chemicals.* 3,4-Dihydroxybenzaldehyde, 2,3-dihydroxybenzaldehyde, 3,4,5-trihydroxybenzaldehyde, 2', 4',6'-trihydroxyacetophenone, phloroglucinol, methoxymethyl chloride and chloroacetonitrile were purchased from Tokyo Kasei Kogyo Co. 2'-Hydroxy-4',6'-dimethoxyacetophenone and 4.0 M HCl solution in dioxane were purchased from Aldrich Chemical Co. 2,2-Diphenyl-1-picrylhydrazyl and other reagents were purchased from Wako Pure Chemical Industries. All solvents used were of reagent grade.

Aparatus. Melting point (mp) data were measured on a hot stage and are uncorrected. Electron ionization mass spectra (EI-MS) were obtained with Jeol JMS-AX500 and JMS-SX102A instruments. NMR spectra were recorded by a Bruker AMX500 spectrometer (<sup>1</sup>H, 500 MHz; <sup>13</sup>C, 125 MHz); chemical shifts are expressed relative to the residual signals of chloroform-d ( $\delta_{\rm H}$  7.26,  $\delta_{\rm C}$  77.0), methanol- $d_4$  ( $\delta_{\rm H}$  3.30,  $\delta_{\rm C}$  49.0) or acetone- $d_6$  $(\delta_{\rm H} 2.04, \delta_{\rm C} 29.8)$ . Optical absorbance was acquired with a Hitachi U-3210 spectrophotometer. Silica gel column chromatography was performed with Wakogel C-300 (Wako Pure Chemical Industries). Normal-phase preparative and analytical thin-layer chromatography (TLC) was performed on silica gel plates, Merck 60 F<sub>254</sub> (0.5 and 0.25 mm thickness, respectively), and reverse phase TLC on RP-18 F<sub>254s</sub> (Merck, 0.2 mm thickness).

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Preparative HPLC was done with an Inertsil PREP-ODS column ( $20.0 \times 250$  mm, GL-Science).

Colorimetric radical scavenging tests. DPPH radical scavenging activity was measured as described previously.<sup>4,5)</sup> To a solution of the DPPH radical (500 µM, 1 ml) in methanol or acetone was added a test solution  $(12.5 \,\mu\text{M}, 4 \,\text{ml})$  in the same solvent in a test tube. The final molar ratio of the radical and test compound was 10:1. The solution was immediately mixed vigorously for 10 s by a Vortex mixer and transferred to a cuvette. The absorbance reading at 517 nm was taken 1, 2, 3, 4, 5, 10, 20 and 30 min after mixing. Acetone and methanol were respectively chosen as aprotic and protic solvents. A solution of dl- $\alpha$ -tocopherol in the same concentration was measured as a positive control. A reduction in the absorbance, 0.228, by the positive control is regarded as corresponding to the consumption of two molecules of the DPPH radical. All experiments were performed in triplicate.

NMR measurement of the reaction mixture of a test compound with the DPPH radical. To the DPPH radical (6.0 equiv, 12.7 µmol) was added a test compound (2.1 µmol) in an acetone- $d_6$  (400 µl) or methanol- $d_4$ acetone- $d_6$  solution (3:1, 400 µl). The methanol-acetone mixture was used as a protic solvent, in which acetone was added as a co-solvent to enhance the solubility of the DPPH radical. The mixture was immediately transferred to an NMR tube and mixed vigorously. <sup>1</sup>H-NMR spectra were recorded 10 min after mixing.

2'-Hydroxy-4',6'-bis(methoxymethoxy)acetophenone<sup>10)</sup> (7). To a mixture of 2',4',6'-trihydroxyacetophenone (168 mg, 1 mmol) and anhydrous K<sub>2</sub>CO<sub>3</sub> (966 mg, 7 mmol) in dry acetone (20 ml) was added dropwise methoxymethyl chloride (20 mg, 2.5 mmol). The mixture was refluxed for 60 min, cooled to room temperature, filtered, and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography (hexane–ethyl acetate (7:3)) to afford 7 (135 mg, 60%) as colorless oil. EI-MS m/z (%): 256 (26, [M]<sup>+</sup>); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 2.65 (3H, s), 3.47, 3.51 (each 3H, s, 2 × OCH<sub>3</sub>), 5.16, 5.25 (each 2H, s, 2 × OCH<sub>2</sub>O), 6.24 (1H, d, J = 2.2 Hz, H-5), 6.26 (1H, d, J = 2.2 Hz, H-3).

3,4-Bis(methoxymethoxy)benzaldehyde (8). By the same procedure as the preparation of 7, 8 was prepared from 3,4-dihydroxybenzaldehyde to give colorless oil (87%): EI-MS m/z (%): 226 (13, [M]<sup>+</sup>); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 3.53 (6H, s, 2 × OCH<sub>3</sub>), 5.30, 5.33 (each 2H, s, 2 × OCH<sub>2</sub>O), 7.29 (1H, dd, J = 7.9, 1.2 Hz, H-6), 7.52 (1H, d, J = 7.9 Hz, H-5), 7.68 (1H, d, J = 1.2 Hz, H-2), 9.87 (1H, s, CHO).

2,3-Bis(methoxymethoxy)benzaldehyde (9). By the same procedure as that used for the preparation of 7, 9

was prepared from 2,3-dihydroxybenzaldehyde to give colorless oil (88%). EI-MS m/z (%): 226 (5, [M]<sup>+</sup>); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 3.52, 3.58 (each 3H, s, 2 × OCH<sub>3</sub>), 5.23, 5.25 (each 2H, s, 2 × OCH<sub>2</sub>O), 7.15 (1H, dd, J = 8.1, 7.8 Hz, H-5), 7.41 (1H, dd, J = 8.1, 1.5 Hz, H-6), 7.51 (1H, dd, J = 7.8, 1.5 Hz, H-4).

3,4,5-Tris(methoxymethoxy)benzaldehyde (10). By the same procedure as that used for the preparation of 7, 10 was prepared from 3,4,5-trihydroxybenzaldehyde to give colorless oil (64%). EI-MS m/z (%): 286 (33, [M]<sup>+</sup>); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 3.52 (6H, s, 2 × OCH<sub>3</sub>), 3.62 (3H, s, OCH<sub>3</sub>), 5.24 (2H, s, OCH<sub>2</sub>O), 5.26 (4H, s, 2 × OCH<sub>2</sub>O), 7.39 (2H, s, H-2 and 5), 9.85 (1H, s, CHO).

2'-Hydroxy-4',6'-dimethoxy-3,4-bis(methoxymethoxy)chalcone (11).<sup>10)</sup> To a stirred solution of KOH (2.0 g. 45.6 mmol) in water (2 ml) cooled to 0 °C in an ice bath was added dropwise a solution of 8 (226 mg, 1.0 mmol) and 2'-hydroxy-4',6'-dimethoxyacetophenone (7a, 294 mg, 1.5 mmol) in methanol (10 ml) under argon. The reaction mixture was kept at 0 °C for 3 h, and then at room temperature for 72 h. The mixture was poured into ice-water (10 ml), adjusted to pH 3-4 with 1 M HCl, and then extracted with ethyl acetate. The organic layer was successively washed with water and saturated brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure to yield a yellow solid. Recrystallization from hexane-ethyl acetate gave 11 as yellow powder (392 mg, 97%). Mp 112–113 °C; EI-HR-MS m/z 404.1450 (calcd. for  $C_{21}H_{24}O_8$ , 404.1471); <sup>1</sup>H-NMR  $(CDCl_3) \delta$  (ppm): 3.52, 3.55, 3.84, 3.92 (each 3H, s,  $4 \times OCH_3$ ), 5.28 (4H, s,  $2 \times OCH_2O$ ), 5.97 (1H, d, J = 2.2 Hz, H-5', 6.11 (1H, d, J = 2.2 Hz, H-3'), 7.18 (1H, d, J = 8.6 Hz, H-5), 7.22 (1H, dd, J = 8.6, 1.9 Hz,H-6), 7.51 (1H, d, J = 1.9 Hz, H-2), 7.73 (1H, d,  $J = 15.5 \text{ Hz}, \text{ H-}\beta), 7.84 (1\text{H}, \text{d}, J = 15.5 \text{ Hz}, \text{H-}\alpha).$ 

2'-Hydroxy-3,4,4',6'-tetrakis(methoxymethoxy)chalcone (12). By the same procedure as the preparation of 11, 12 was prepared from 7 and 8. The residue was purified by silica gel column chromatography (hexaneethyl acetate (7:3)) to give 12 as yellow powders (74%): Mp 87–88 °C; EI-HR-MS m/z 464.1691 (calcd. for C<sub>23</sub>H<sub>28</sub>O<sub>10</sub>, 464.1682); <sup>1</sup>H-NMR (acetone- $d_6$ )  $\delta$  (ppm): 3.45, 3.47, 3.50, 3.56 (each 3H, s, 4 × OCH<sub>3</sub>), 5.26, 5.27, 5.28, 5.42 (each 2H, s, 4 × OCH<sub>2</sub>O), 6.24 (1H, d, J = 2.2 Hz, H-5'), 6.34 (1H, d, J = 2.2 Hz, H-3'), 7.20 (1H, d, J = 8.6 Hz, H-5), 7.34 (1H, dd, J = 8.6, 2.2 Hz, H-6), 7.56 (1H, d, J = 2.2 Hz, H-2), 7.74 (1H, d, J = 15.8 Hz, H- $\beta$ ), 7.96 (1H, d, J = 15.8 Hz, H- $\alpha$ ).

2'-Hydroxy-4',6'-dimethoxy-2,3-bis(methoxymethoxy)chalcone (13). By the same procedure as that used for the preparation of 11, 13 was prepared from 7a and 9 to give yellow powder (88%). Mp 94–95 °C; EI-HR-MS m/z 404.1440 (calcd. for C<sub>21</sub>H<sub>24</sub>O<sub>8</sub>, 404.1471); <sup>1</sup>H- NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 3.51, 3.64, 3.83, 3.90 (each 3H, s, 4 × OCH<sub>3</sub>), 5.19, 5.22 (each 2H, s, 2 × OCH<sub>2</sub>O), 5.96 (1H, d, J = 2.3 Hz, H-5'), 6.11 (1H, d, J = 2.3 Hz, H-3'), 7.08 (1H, dd, J = 8.1, 7.9 Hz, H-5), 7.19 (1H, dd, J = 8.1, 1.5 Hz, H-6), 7.33 (1H, dd, J = 7.9, 1.5 Hz, H-4), 7.90 (1H, d, J = 15.8 Hz, H- $\beta$ ), 8.20 (1H, d, J = 15.8 Hz, H- $\alpha$ ).

2'-Hydroxy-2,3,4',6'-tetrakis(methoxymethoxy)chalcone (14). By the same procedure as that used for the preparation of 11, 14 was prepared from 7 and 9 and purified by silica gel column chromatography (hexane– ethyl acetate (7:3)) to give yellow powder (440 mg, 95%). Mp 67–68 °C; EI-HR-MS m/z 464.1682 (calcd. for C<sub>23</sub>H<sub>28</sub>O<sub>10</sub>, 464.1682); <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ (ppm): 3.48, 3.51, 3.52, 3.64 (each 3H, s, 4 × OCH<sub>3</sub>), 5.19, 5.19, 5.22, 5.29 (each 2H, s, 4 × OCH<sub>2</sub>O), 6.26 (1H, d, J = 2.5 Hz, H-5'), 6.32 (1H, d, J = 2.5 Hz, H-3'), 7.08 (1H, dd, J = 8.1, 7.9 Hz, H-5), 7.20 (1H, dd, J = 8.1, 1.2 Hz, H-6), 7.32 (1H, dd, J = 7.9, 1.2 Hz, H-4), 7.92 (1H, d, J = 15.8 Hz, H- $\beta$ ), 8.22 (1H, d, J = 15.8 Hz, H- $\alpha$ ).

2'-Hydroxy-4',6'-dimethoxy-3,4,5-tris(methoxymethoxy)chalcone (15). By the same procedure as that used for the preparation of 11, 15 was prepared from 7a and 10 to give yellow powder (64%). Mp 110–111 °C; EI-HR-MS m/z 464.1635 (calcd. for C<sub>23</sub>H<sub>28</sub>O<sub>10</sub>, 464.1682); <sup>1</sup>H-NMR (acetone- $d_6$ ) δ (ppm): 3.51 (6H, s, 2 × OCH<sub>3</sub>), 3.57, 3.87, 4.00 (each 3H, s, 3 × OCH<sub>3</sub>), 5.15 (2H, s, OCH<sub>2</sub>O), 5.30 (4H, s, 2 × OCH<sub>2</sub>O), 6.09 (1H, d, J =2.2 Hz, H-5'), 6.12 (1H, d, J = 2.2 Hz, H-3'), 7.24 (2H, s, H-2 and 6), 7.66 (1H, d, J = 15.5 Hz, H- $\beta$ ), 7.94 (1H, d, J = 15.5 Hz, H- $\alpha$ ).

2'-Hydroxy-3,4,4',5,6'-pentakis(methoxymethoxy)chalcone (16). By the same procedure as that used for the preparation of 11, 16 was prepared from 7 and 10 and purified by silica gel column chromatography (hexaneethyl acetate (7:3)) to give yellow powder (65%). Mp 89–90 °C; EI-HR-MS m/z 524.1908 (calcd. for  $C_{25}H_{32}O_{12}$ , 524.1894); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  (ppm): 3.48 (3H, s, OCH<sub>3</sub>), 3.51 (6H, s, 2 OCH<sub>3</sub>), 3.54, 3.62 (each 3H, s, OCH<sub>3</sub>), 5.19, 5.19 (each 2H, s, 2 × OCH<sub>2</sub>O), 5.23 (4H, s, 2 × OCH<sub>2</sub>O), 5.30 (2H, s, OCH<sub>2</sub>O), 6.28 (1H, d, J = 2.5 Hz, H-5'), 6.31 (1H, d, J = 2.5 Hz, H-3'), 7.16 (2H, s, H-2 and 6), 7.69 (2H, d, J = 15.5 Hz, H- $\beta$ ), 7.86 (1H, d, J = 15.5 Hz, H- $\alpha$ ).

2',3,4-Trihydroxy-4',6'-dimethoxychalcone<sup>10)</sup> (1). To a stirred solution of **11** (101 mg, 0.25 mmol) in methanol (5 ml) was added dropwise 3 M HCl (2 ml). The mixture was refluxd for 45 min, diluted with water (5 ml), and extracted with ethyl acetate. The organic layer was successively washed with water and brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (chloroform–methanol (100:3)) to give **1** as yellow powder (53 mg, 67%). Mp 174–175 °C; EI-HR-MS m/z 316.0926 (calcd. for C<sub>17</sub>H<sub>16</sub>O<sub>6</sub>, 316.0947); <sup>1</sup>H-NMR (acetone- $d_6$ )  $\delta$  (ppm): 3.86, 4.00 (each 3H, s, 2 × OCH<sub>3</sub>), 6.08 (1H, d, J = 2.5 Hz, H-5'), 6.11 (1H, d, J = 2.5 Hz, H-3'), 6.89 (1H, d, J = 8.1 Hz, H-5), 7.11 (1H, dd, J = 8.1, 2.0 Hz, H-6), 7.24 (1H, d, J = 2.0 Hz, H-2), 7.69 (1H, d, J = 15.5 Hz, H- $\beta$ ), 7.83 (1H, d, J =15.5 Hz, H- $\alpha$ ).

2',3,4,4',6'-Pentahydroxychalcone (2). By the same procedure as that used for the preparation of **1**, **2** was prepared from **12** and purified by silica gel column chromatography (chloroform–methanol (9:1)) to give yellow powder (41%). Mp 157–158 °C (lit.<sup>11)</sup> 249– 252 °C); EI-HR-MS *m*/*z* 288.0602 (calcd. for C<sub>15</sub>H<sub>12</sub>O<sub>6</sub>, 288.0634); <sup>1</sup>H-NMR (acetone-*d*<sub>6</sub>)  $\delta$  (ppm): 5.95 (2H, s, H-3' and 5'), 6.88 (1H, d, *J* = 8.1 Hz, H-5), 7.07 (1H, dd, *J* = 8.1, 1.8 Hz, H-6), 7.19 (1H, d, *J* = 1.8 Hz, H-2), 7.69 (1H, d, *J* = 15.5 Hz, H- $\beta$ ), 8.05 (1H, d, *J* = 15.5 Hz, H- $\alpha$ ). The structure was confirmed by satisfactory matching of the analytical data compared the reference data, although melting point was significantly lower than the reference figure.

2,2',3-*Trihydroxy-4'*,6'-*dimethoxychalcone* (3). By the same procedure as that used for the preparation of **1**, **3** was prepared from **13** to give yellow powder (60%). Mp 175–176 °C; EI-HR-MS m/z 316.0918 (calcd. for C<sub>17</sub>H<sub>16</sub>O<sub>6</sub>, 316.0947); <sup>1</sup>H-NMR (acetone-*d*<sub>6</sub>)  $\delta$  (ppm): 3.83, 3.94 (each 3H, s, 2 × OCH<sub>3</sub>), 6.04 (1H, d, J = 2.4Hz, H-5'), 6.07 (1H, d, J = 2.4 Hz, H-3'), 6.68 (1H, dd, J = 8.1, 7.2 Hz, H-5), 6.87 (1H, d, J = 7.2 Hz, H-6), 7.11 (1H, d, J = 8.1 Hz, H-4), 8.12 (2H, m, H- $\alpha$  and  $\beta$ ).

2,2',3,4',6'-Pentahydroxychalcone (4). By the same procedure as that used for the preparation of 1, 4 was prepared from 14 and purified by silica gel column chromatography (chloroform–methanol (9:1)) to give yellow powder (12%). Mp 166–167 °C; EI-HR-MS m/z 288.0627 (calcd. for C<sub>15</sub>H<sub>12</sub>O<sub>6</sub>, 288.0634); <sup>1</sup>H-NMR (acetone- $d_6$ )  $\delta$  (ppm): 5.96 (2H, s, H-3' and 5'), 6.72 (1H, t, J = 7.9 Hz, H-5), 6.89 (1H, dd, J = 7.9, 1.3, H-6), 7.15 (1H, dd, J = 7.9, 1.3 Hz, H-4), 8.21 (1H, d, J = 15.6 Hz, H- $\beta$ ), 8.28 (1H, d, J = 15.6 Hz, H- $\alpha$ ).

2',3,4,5,-*Tetrahydroxy*-4',6'-*dimethoxychalcone* (5). By the same procedure as that used for the preparation of **1**, **5** was prepared from **15** to give yellow powder (25%). Mp 163–164 °C; EI-HR-MS m/z 332.0919 (calcd. for C<sub>17</sub>H<sub>16</sub>O<sub>7</sub>, 332.0896); <sup>1</sup>H-NMR (acetone- $d_6$ ) δ (ppm): 3.86, 4.00 (each 3H, s, 2 × OCH<sub>3</sub>), 6.07 (1H, d, J = 2.4 Hz, H-5'), 6.11 (1H, d, J = 2.4 Hz, H-3'), 6.81 (2H, s, H-2 and 6), 7.61 (1H, d, J = 15.5 Hz, H- $\beta$ ), 7.80 (1H, d, J = 15.5 Hz, H- $\alpha$ ).

2',3,4,4',5,6'-Hexahydroxychalcone (6). By the same procedure as that used for the preparation of 1, 6 was prepared from 16 and purified by silica gel column







Scheme 1. Synthesis of 1–6. Reagents and conditions: (a) KOH, methanol, stirred for 72 h, rt.; (b) 3 M HCl, methanol, refluxed for 45 min.

chromatography (chloroform–methanol (9:1)) to give yellow powders (11%). Mp 179–180 °C; EI-HR-MS m/z304.0566 (calcd. for C<sub>15</sub>H<sub>12</sub>O<sub>7</sub>, 304.0583); <sup>1</sup>H-NMR (acetone- $d_6$ )  $\delta$  (ppm): 5.95 (2H, s, H-3' and 5'), 6.77 (2H, s, H-2 and 6), 7.61 (1H, d, J = 15.6 Hz, H- $\beta$ ), 8.03 (1H, d, J = 15.6 Hz, H- $\alpha$ ).

2-Chloro-2',4',6'-trihydroxyacetophenone<sup>12)</sup> (17). To a mixture of phloroglucinol (5.0 g, 39.7 mmol) and chloroacetonitrile (2.5 ml, 39.7 mmol) in dioxane (20 ml) was added anhydrous ZnCl<sub>2</sub> (0.54 g, 3.96 mmol). The solution was cooled to 0 °C, and a 4.0 M HCl solution in dioxane (60 ml) was added to it. The mixture was left overnight, and further 4.0 M HCl in dioxane (20 ml) was added to it. The precipitated imine was collected by filtration and washed three times with ether. The imine was dissolved in 100 ml of 1 M HCl and heated at 100 °C for 1 h. The solid was collected by filtration, washed three times with water and dried under vacuum to yield a pure acetophenone (17) as a pale white solid which was used without further purification (2.2 g, 28%). EI-MS m/z (%): 202 (26, [M]<sup>+</sup>).

4,6-Dihydroxybenzofuran-3(2H)-one<sup>12</sup>) (18). To a solution of 17 (2.2 g, 11.1 mmol) in methanol (50 ml) was added NaOMe (1.6 g, 29.3 mmol), and the mixture was refluxed for 2 h. After cooling, the solution was acidified with 1 M HCl and evaporated under reduced pressure. The resulting residue was extracted with ethyl acetate, and the extract was successively washed with

water and brine, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to give pure **18** (1.3 g, 71%). EI-MS m/z (%): 166 (100,  $[M]^+$ ).

Aureusidin<sup>12,13</sup> (19). To a solution of 18 (100 mg, 0.6 mmol) and 3,4-dihydroxybenzaldehyde (83 mg, 0.6 mmol) in acetic acid (5 ml) was added conc. HCl (0.2 ml). After stirring for 5 hr at room temperature, the solution was poured into water and extracted with ethyl acetate. The ethyl acetate solution was dried with anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated under reduced pressure. The residue was subjected to preparative HPLC (ODS, methanol-water-formic acid (60:40:0.1); 5.0 ml/min flow rate; UV 254 nm detection) to give 19  $(t_{\rm R} 14 \,\mathrm{min})$  as yellow powder  $(12 \,\mathrm{mg}, 7\%)$ . Mp 246– 248 °C; EI-HR-MS m/z 286.0507 (calcd. for C<sub>15</sub>H<sub>10</sub>O<sub>6</sub>, 286.0477); <sup>1</sup>H-NMR (methanol- $d_4$ )  $\delta$  (ppm): 6.02 (1H, brs, H-7), 6.20 (1H, brs, H-5), 6.56 (1H, s, H-α), 6.82 (1H, d, J = 8.1 Hz, H-5'), 7.18 (1H, brd, 8.1 Hz, H-6'),7.47 (1H, brs, H-2').

4,6-Dimethoxybenzofuran-3(2H)-one<sup>13)</sup> (20). To a solution of **18** (332 mg, 2.0 mmol) in DMF (10 ml) was added K<sub>2</sub>CO<sub>3</sub> (0.55 g, 4.0 mmol) and MeI (0.37 ml, 6.0 mmol). The solution was heated at 80 °C for 1 h, poured into water and extracted with ethyl acetate. The ethyl acetate solution was dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated under reduced pressure to yield brown viscous oil. This oil was subjected to silica gel column chromatography (hexane–ethyl acetate (1:1)) to

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give **20** as yellow powder (290 mg, 75%). EI-MS m/z (%):194 (100, [M]<sup>+</sup>).

4,6-Di-O-methylaureusidin (21). By the same procedure as that used for the preparation of 19, 21 was prepared from 20 and 3,4-dihydroxybenzaldehyde as yellow powder (94%). Mp 170–171 °C; EI-HR-MS m/z314.0800 (calcd. for C<sub>17</sub>H<sub>14</sub>O<sub>6</sub>, 314.0790); <sup>1</sup>H-NMR (methanol- $d_4$ )  $\delta$  (ppm): 3.92, 3.93 (each 3H, s, 2 × OCH<sub>3</sub>), 6.28 (1H, d, J = 1.7 Hz, H-7), 6.53 (1H, d, J =1.7 Hz, H-5), 6.62 (1H, s, H- $\alpha$ ), 6.82 (1H, d, J = 8.4 Hz, H-5'), 7.22 (1H, dd, J = 8.4, 2.0 Hz, H-6'), 7.47 (1H, d, J = 2.0 Hz, H-2').

Eriodictyol<sup>14)</sup> (22). To a solution of 2 (432 mg, 1.5mmol) in methanol (50 ml) was added KF (100 mg, 1.7 mmol). The solution was refluxed for 24 h. The reaction mixture was diluted with water (50 ml) and extracted with ethyl acetate. The resulting ethyl acetate extract was dried with anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated under reduced pressure. The resulting residue was subjected to HPLC (ODS, methanol-water-formic acid (70:30:0.1); 5.0 ml/min flow rate; UV 254 nm detection), to give 22 ( $t_{\rm R}$  12 min) as pink powder (173 mg, 40%). Mp 240-242 °C (lit.<sup>15)</sup> 265-266 °C); EI-HR-MS 288.0673 (calcd. for  $C_{15}H_{12}O_6$ , 288.0634); <sup>1</sup>H-NMR (methanol- $d_4$ )  $\delta$  (ppm): 2.69 (1H, dd, J = 17.0, 3.0 Hz,H-3), 3.06 (1H, dd, J = 17.0, 12.5 Hz, H-3), 5.27 (1H, dd, J = 12.5, 3.0 Hz, H-2), 5.87 (1H, d, J = 2.3 Hz, H-8), 5.89 (1H, d, J = 2.3 Hz, H-6), 6.78–6.91 (3H, m, H-2′,5′,6′).

#### **Results and Discussion**

Chalcones **1–6** were prepared by condensation of the corresponding acetophenones and benzaldehydes (Scheme 1). Time-course characteristics for the radical scavenging activity of **1–6** in acetone and methanol are shown in Fig. 1. At 30 min, the relative radical scavenging equivalence of each compound, when that of  $\alpha$ tocopherol in ethanol as a standard is designated as 2, was as follows: **1**, 2.1; **2**, 3.8; **3**, 1.6; **4**, 3.4; **5**, 4.4; **6**, 4.6 in acetone; and **1**, 5.8; **2**, 4.0; **3**, 4.8; **4**, 3.9; **5**, 5.6; **6**, 5.1 in methanol.

Compound 1 has a 2'-hydroxy-4',6'-dimethoxylated A-ring and 3,4-dihydroxylated B-ring, whereas 2 has a 2',4',6'-trihydroxylated A-ring and the same B-ring as 1. Interestingly, 1 showed a dramatic increase in DPPH radical consumption when changing the solvent from aprotic acetone to protic methanol, while 2 showed little difference change in radical scavenging activity in eiter solvent. The reaction mixture of 1 (or 2) with DPPH radical in acetone- $d_6$  (or methanol- $d_4$ ) was directly analyzed by <sup>1</sup>H-NMR. In the <sup>1</sup>H-NMR spectrum of the reaction mixture of 1 with the DPPH radical in acetone- $d_6$  (Fig. 2A), characteristic signals of a 4-substituted *o*-quinone ring at  $\delta$  6.50 (d, J = 10.3 Hz),  $\delta$  6.68 (brs) and 7.69 (dd, J = 10.3, 1.7 Hz), *trans*-double bond protons



**Fig. 1.** DPPH Radical Scavenging Activity of  $1 (\bigcirc), 2 (\bigcirc), 3 (\triangle), 4 (\blacktriangle), 5 (\Box)$  and  $6 (\blacksquare)$  in Acetone (A) and in Methanol (B).

at 7.50 (d, J = 15.5 Hz) and  $\delta$  8.09 (d, J = 15.5 Hz), and two *meta*-coupled doublet peaks of  $\delta$  6.12 (d, J =2.0 Hz) and 6.14 (d, J = 2.0 Hz) were observed. These signals are assignable to the corresponding quinone, **1q**. In addition, there appeared minor signals of  $\delta$  6.45 (d, J = 10.1), 6.39, 6.41 and 6.92 (each s), and  $\delta$  7.78 (dd, J = 10.1, 1.7 Hz). It is suggested that this latter set of signals was due to a ring-closed aurone derivative. Hence, the corresponding aurone, 4,6-di-*O*-methylaureusidin (**21**) was synthesized according to the procedure shown in Scheme 2, and oxidized with the DPPH radical for comparison. In the <sup>1</sup>H-NMR spectrum of the reaction mixture of **21** and the DPPH radical in acetone- $d_6$ (Fig. 2B), the same set of signals appeared as that in the mixture of **1** and the DPPH radical.

The <sup>1</sup>H-NMR analysis indicated the following reaction of 1 in acetone: Compound 1 scavenges two equivalents of the DPPH radical and is converted into



Fig. 2. <sup>1</sup>H NMR Spectra of the Reaction Mixtures of 1 (A) and 21 (B) with the DPPH Radical in Acetone- $d_6$ .



Scheme 2. Synthesis of 19, 21 and 22.

Reagents and conditions: (a) i.  $ZnCl_2$ , dioxane, HCl–dioxane solution; ii. 1 N HCl, 100 °C, 1 h; (b) MeONa, methanol, refluxed for 2 h; (c) 3,4-dihydroxybenzaldehyde, HOAc, conc. HCl, stirred for 5 h, rt.; (d) MeI, K<sub>2</sub>CO<sub>3</sub>, DMF, 80 °C, 1 h; (e) KF, methanol, refluxed for 24 h.



Fig. 3. Plausible Oxidation Mechanism of 2',3,4-Trihydroxy-4',6'-dimethoxychalcone (1) by the DPPH Radical.

the corresponding quinone, 1q. The A-ring of 1q then slowly rotates to yield a 1q' conformer, by which intramolecular addition of a phenolic OH on the A-ring to an  $\alpha$ -carbon of the bridged enone and successive deprotonation gives aurone 21. Resulting 21 can scavenge two more DPPH radicals and is converted into 21q (Fig. 3). Although 21q was detected in the <sup>1</sup>H-NMR spectrum of the reaction mixture, the DPPH radical scavenging equivalence of 1 at 30 min was 2.1 in acetone, and hence, contribution of the formation of 21q to the antiradical efficiency of 1 might be limited.

In contrast, the <sup>1</sup>H-NMR spectrum of the reaction mixture of between **1** and the DPPH radical in methanol gave a considerably complex pattern composed of many small peaks (data not shown). By taking into account nearly six DPPH radical consumption of **1** in methanol, the intermediate quinone (**1q**) produced was quickly cyclized to yield aurone **21** and then its quinone **21q**, which might be further oxidized to a complex mixture. The higher reaction rate of **1** in methanol compared to that in acetone was probably due to solvation of the Aring hydroxyl and carbonyl groups, which would reduce the intramolecular hydrogen bond between them and favor the rotation of the A-ring to a suitable position for cyclization to aurone **21**.

Figure 4A shows the <sup>1</sup>H-NMR spectrum of the reaction mixture of **2** with the DPPH radical in acetone- $d_6$ . The Lack of signals of the *trans*-double bond protons indicats that **2** was readily oxidized and

cyclized by the DPPH radical to give ring closure products. Therefore, the corresponding aurone (aureusidin, 19) and flavanone (eriodictyol, 22) were synthesized according to the procedure shown in Scheme 2, respectively, for comparison. The <sup>1</sup>H-NMR spectra of the reaction mixtures of 19 and 22 with the DPPH radical in acetone- $d_6$  are shown in Fig. 4B and 4C, respectively. In Fig. 4B, signals characteristic of aurone quinone **19q** were observed, which are supported by comparing with the signals in Fig. 2B. The higher field shift of H- $\alpha$ ,  $\delta$  6.42, in **19q** compared with  $\delta$  6.68 in **21q** can be accounted for by the presence of an intramolecular hydrogen bond in 19q, which would reduce the electronwithdrawing nature of the ketone conjugated with an exocyclic double bond. In Fig. 4C, the characteristic doublet and doublet of doublet signals of H-5' and 6' in corresponding **22q** at  $\delta$  6.48 (d, J = 10.0 Hz) and 7.42 (dd, J = 10.0, 2.0 Hz), respectively, and a broad singlet peak at  $\delta$  6.53 assignable to H-2' are shown, together with A-ring protons of  $\delta$  6.00 and 6.07. All of the signals found in Fig. 4B and 4C also exist in Fig. 4A, which suggests that 2 was readily oxidized by DPPH radical and spontaneously converted to both 19q and 22q.

These results indicate the reaction of 2 in acetone: Compound 2 scavenges two equivalents of the DPPH radical and is converted to 2q. Resulting 2q cyclizes more readily than 1q, since free 6'-OH exists in 2q in place of 6'-OMe in 1q. When an intramolecular Michael-type addition occurs on C- $\beta$ , regeneration of



Fig. 4. <sup>1</sup>H NMR Spectra of the Reaction Mixtures of 2 (A), 19 (B) and 22 (C) with the DPPH Radical in Acetone-d<sub>6</sub>.

the C-4 ketone would directly give 22q. However, if cyclization occurs on C- $\alpha$ , re-aromatization of the Bring by deprotonation at C- $\beta$  would yield catechol 19, which could spontaneously undergo oxidation by consuming two equivalents of the DPPH radical and convert to its quinone, 19q. The peak area ratio of the cyclization products in Fig. 4A indicats that the intramolecular attack occurred preferentially on C- $\alpha$  to give aurone quinone 19q, rather than 22q. This characteristic is in accordance with the radical scavenging activity of 2, reaching four equivalents. Unexpectedly, the radical scavenging reaction of 2 in methanol was basically the same as that in acetone. An analysis of <sup>1</sup>H-NMR data from the reaction mixture of 2 with the DPPH radical in methanol suggests that 2 was oxidized by the DPPH radical and converted to 22q and 19q (data not shown) as shown with the reaction in acetone. It is still unclear why 19q did not undergo further oxidation which might have been triggered by nucleophilic addition of a methanol molecule to the B-ring quinone<sup>2)</sup> unlike 21q. The presence of an intramolecular hydrogen bond in **19**q could reduce the electron-withdrawing property of the ketone as shown by the chemical shift difference in H- $\alpha$ of **19q** ( $\delta$  6.42) and **21q** ( $\delta$  6.68) just described. Thus, the electron density of the quinone ring would be higher in 19q than that in 21q, which would be unfavorable for undergoing nucleophilic attack (Fig. 5).

Compounds 3 and 4 are the 2,3-dihydroxylated analogues of 1 and 2, respectively. In acetone, the radical scavenging equivalence of 3 is 1.6, and of 4 is 3.4, while in methanol, the figures are 4.8 and 3.9,

respectively. In each case, the activity was lower than that of 1 and 2. This result suggests that 3 and 4 were reluctantly oxidized and converted into 3q and 4qcompared to the rapid oxidation of 1 and 2. The slower conversion rate to their quinones might have resulted in reduction of the total radical scavenging activity, although partial cyclization occurred from 3q and 4q.

In contrast, pyrogallol analogues 5 and 6 showed similar DPPH radical consumption of 4.4 and 4.6 in acetone, and of 5.6 and 5.1 in methanol, respectively. It is known that the pyrogallol-type phenolic acid, gallic acid, consumed more than four DPPH radicals in both aprotic and protic solvents.<sup>5)</sup> In addition, the oxidized galloquinone has a tendency to dimerize, since an additional hydroxyl group has an electron-releasing nature; thus, the quinone could act as a nucleophile to add another molecule of quinone. This easy intermolecular-coupling nature complicates the whole oxidation reaction of pyrogallols and may be a cause of their enhanced radical scavenging efficiency. However, the detailed reaction scheme is still unclear, since NMR profiles of the reaction mixtures of 5 and 6 with the DPPH radical were complicated and difficult to fully analyze.

In summary, the radical scavenging activity of polyhydroxychalcones was dependent on their hydroxylation pattern. Concerning the B-ring, pyrogallol-type 3,4,5-trihydroxylated chalcones showed higher activity than the catechol-type 3,4-dihydroxylated analogues, whereas the activity of the 2,3-dihydroxylated isomers was low. This trend is comparable to those found in



Fig. 5. Plausible Oxidation Mechanism for 2',3,4,4',6'-Pentahydroxychalcone (2) by the DPPH Radical.

other flavonoids and catechines. Interestingly, methyl etherification of the A-ring hydroxyls in 3,4-hydroxylated chalcones greatly affected their antiradical efficiency. This phenomenon might be accounted for by a difference in the readiness for cyclization of the chalcone quinones to aurones and/or flavanones and by intramolecular hydrogen bonding between an A-ring hydroxyl and a C-ring carbonyl in the resulting cyclized quinone intermediates.

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