**DPPH Radical-scavenging Effect of Several Phenylpropanoid Compounds and Their Glycoside Derivatives**

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Eugenol, isoeugenol, caffeic acid, ferulic acid, isoferrulic acid, estragole, trans-anethole, and paeonol are components of a Chinese herbal medicine used as a painkiller and stomachic. We investigated the potential role of these compounds as antioxidants. We studied the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging effect of these molecules, together with some glycoside derivatives, to ascertain their potential in reducing the levels of activated oxygen species in vivo. The DPPH radical-scavenging effects of eugenol, isoeugenol, and the glycoside derivatives of caffeic acid, ferulic acid, and isoferrulic acid (SC50=8–28 μM) were similar to those of α-tocopherol, which was used as a positive control.

**Key words**—DPPH (1,1-diphenyl-2-picrylhydrazyl) radical; active oxygen; radical-scavenging effect; phenylpropanoid glycoside

**INTRODUCTION**

Antioxidants, which scavenge active oxygen species (free radicals), are found in a variety of foodstuffs and are commonly referred to as scavengers. Many antioxidants are plant based and play an important role in protecting plants that are exposed to strong sunlight and live under severe oxygen stress. Antioxidants also play an important role in human health because the biologic defense mechanisms cannot operate under severe oxygen stress. According to recent research, activated oxygen is thought to be a major factor in aging, hardening of the arteries, diabetes, cancer, and tissue injury skin. Indeed, approximately 90% of age-related diseases are linked to activated oxygen.

When human skin is exposed to ultraviolet rays (UV-B) active oxygen (free radical) is generated, which is scavenged by excess melanin. Pigmentation from excess melanin can cause the appearance of spots and freckles on the skin. Furthermore, when human skin is exposed to ultraviolet rays (UV-A) the radiation penetrates the surface and is absorbed by the inner layers of the skin. The generation of active oxygen in these inner layers can destroy components between the cells, causing wrinkles and skin flaps. We searched for antioxidants that are effective in scavenging active oxygen species. Eight phenylpropanoid compounds, which are components of botanic essential oils, were easily obtained. In addition, the glycoside derivatives were synthesized. Their scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals was tested and the most effective antioxidants for scavenging oxygen free radicals were identified.

**EXPERIMENTAL**

**Materials** Eugenol (4-allyl-2-methoxyphenol) 1, isoeugenol [2-methoxy-4-(1-propenyl) phenol] 2, caffeic acid (3,4-dihydroxycinnamic acid) 3, ferulic acid (4-hydroxy-3-methoxycinnamic acid) 4, isoferrulic acid (3-hydroxy-4-methoxycinnamic acid) 5, estragole (4-allylanisole) 6, trans-anethole [trans-1-methoxy-4-(1-propenyl) benzene] 7, paeonol 8, and α-tocopherol 9 were obtained from Tokyo Kasei Kougyou Co.

**Analysis** The structures of products: 1a, 2a, 3a, 4a, and 5a were established based on the 1H-NMR (400 MHz) and MS spectra.

**Synthesis**

**Synthesis of Glycoside** When a reaction was shown, a solution of 3,4-diacyltycinnamic acid (0.240 g, 1.0×10⁻³ mol) and 1,1,3,3-tetramethylurea (0.2 g, 2.0×10⁻³ mol) was poured into 10 mL of 1,1-diphenyl-2-picrylhydrazyl (DPPH, 1×10⁻⁵ mol) in ethanol. The mixture was stirred for 10 minutes and the absorbance was determined. The absorbance was compared with the control (DPPH was not added).
ml, $1.7 \times 10^{-3}$ mol in CH$_2$Cl$_2$ (dehydrated, 5 ml) was added, and the mixture was stirred with aceto- bromo-$\alpha$-glucose (0.617 g, $1.5 \times 10^{-3}$ mol) and stannous triflate (0.625 g, $1.5 \times 10^{-3}$ mol in CH$_2$Cl$_2$ (5 ml) at room temperature (20±2°C). After 18 h, aqueous NaHCO$_3$ solution (100 ml) was added the solution, which was then extracted twice with CH$_2$Cl$_2$ (100 ml). The extract was washed with saturated aqueous NaCl, dried over anhydrous Na$_2$SO$_4$, evaporated under reduced pressure, purified by column chromatography on silica gel with hexane-EtOAc (1 : 1) to afford 3,4-diacyctylaminic acid tetracetylglycoside, 0.601 g (1.2 $\times 10^{-3}$ mol, 60.4%). After deacetylation, a solution of 3,4-diacyctylaminic acid tetracetylglycoside 0.233 g (5 $\times 10^{-4}$ mol) in CH$_2$OH-THF (1 : 1, 10 ml) was placed in CH$_2$ONa and neutralized with ion-exchange resin (Dowex-50W). After evaporation under reduced pressure, the residue was refined by silica gel chromatography with CHCl$_3$-CH$_2$OH (5 : 1) to afford 3,4-dihydroxyaminic acid glucopyranoside 3a 0.025 g (2.9 $\times 10^{-3}$ mol, 15%).

This procedure was repeated for compounds 1, 2, 4, and 5 : 1 to citrusin C 1a (yield 94.1%), 2 to isocitrusin C 2a (yield 82.1%), 4 to 3-methoxy-4-hydroxyaminic acid glucopyranoside 4a 0.045 g (yield 25.3%), and 5 to 3-hydroxy-4-methoxyaminic acid glucopyranoside 5a 0.064 g (yield 36.2%).

1a Yield: 94.1%, [α]$_D$ $^{20}$ = +46.60°, m.p. 132–133.2°C.

1H-NMR $\delta$CD$_2$OD (ppm): 3.31 (2H, d, $J$=6.5 Hz, H-7), 3.68 (1H, dd, $J$=4.8, 12.1, H-6′α), 3.83 (3H, s, -OCH$_3$), 3.87 (1H, d, $J$=11.9, H-6′b), 4.84 (1H, d, $J$=7.4, H-1′α), 5.04 (1H, dd, $J$=1.6, 10.1 Hz, H-9α), 5.05 (1H, dd, $J$=1.6, 17.4 Hz, H-9b), 5.95 (1H, dd, $J$=6.5, 14.2 Hz, H-8), 6.72 (1H, d, $J$=1.6, 8.4 Hz, H-5), 6.82 (1H, d, $J$=1.6 Hz, H-3), 7.08 (1H, d, $J$=8.4 Hz, H-6).

MS m/z (relative abundance %): 327 ([M+H]$^+$, 5), 325 (17), 165 (64), 164 (100), 163 (22), 145 (21), 127 (9), 85 (18), 73 (9), 57 (8).

2a Yield: 82.1%, [α]$_D$ $^{20}$ = -42.84°, m.p. 129–131.5°C.

1H-NMR $\delta$CD$_3$Cl (ppm): 3.84 (3H, s, -OCH$_3$), 6.03 (1H, dt, $J$=14.5, 6.0 Hz, H$_2$), 6.20 (1H, dt, $J$=14.5, 2.8 Hz, H$_3$), 6.76 (1H, dd, $J$=8.6, 3.2 Hz, H$_2$), 6.82 (1H, d, $J$=2.6 Hz, H$_3$), 6.99 (1H, d, $J$=7.0 Hz, H$_2$).

MS m/z (relative abundance %): 327 ([M+H]$^+$, 6), 325 (11), 165 (56), 164 (100), 163 (19), 145 (39), 127 (6), 85 (22), 73 (11), 57 (16).

3a Yield: 15.0%, [α]$_D$ $^{20}$ = -34.31°, m.p. 181–182°C.

1H-NMR $\delta$CD$_3$Cl (ppm): 3.33 (1H, m, Glc-4H), 4.21 (1H, d, $J$=10.5 Hz, Glc-6H), 4.41 (1H, m, Glc-5H), 5.31 (1H, m, Glc-3H), 5.37 (1H, m, Glc-2H), 5.89 (1H, d, $J$=8.1 Hz, Glc-1H), 6.41 (1H, d, $J$=8.6 Hz, -CH=CHCOO-), 7.16 (1H, d, $J$=16.1 Hz, ph-H), 7.30 (1H, d, $J$=8.6 Hz, -CH=CHCOO-), 7.52 (1H, s, ph-H), 7.58 (1H, d, $J$=16.1 Hz, ph-H).

MS m/z (relative abundance %): 343 ([M+H]$^+$, 15), 341 (70), 325 (74), 311 (68), 275 (73), 183 (100), 179 (38), 91 (97), 89 (71), 59 (71).

4a Yield: 25.3%, [α]$_D$ $^{20}$ = -21.28°, m.p. 172–174°C.

1H-NMR $\delta$CD$_3$Cl (ppm): 3.14 (1H, m, Glc-4H), 3.76 (3H, s, -OCH$_3$), 4.10 (1H, d, $J$=10.5 Hz, Glc-6H), 4.37 (1H, m, Glc-5H), 5.16 (1H, m, Glc-3H), 5.34 (1H, m, Glc-2H), 5.79 (1H, d, $J$=8.1 Hz, Glc-1H), 6.44 (1H, d, $J$=8.6 Hz, -CH=CHCOO-), 7.21 (1H, d, $J$=16.1 Hz, ph-H), 7.29 (1H, d, $J$=8.6 Hz, -CH=CHCOO-), 7.53 (1H, s, ph-H), 7.68 (1H, d, $J$=16.1 Hz, ph-H).

MS m/z (relative abundance %): 357 ([M+H]$^+$, 9), 356 (28), 355 (46), 325 (8), 311 (5), 275 (60), 183 (100), 179 (8), 91 (87), 89 (21), 59 (16).

5a Yield: 36.2%, [α]$_D$ $^{20}$ = -25.29°, m.p. 176–178°C.

1H-NMR $\delta$CD$_3$Cl (ppm): 3.26 (1H, m, Glc-4H), 3.75 (3H, s, ph-OCH$_3$), 4.18 (1H, d, $J$=10.5 Hz, Glc-6H), 4.32 (1H, m, Glc-5H), 5.19 (1H, m, Glc-3H), 5.41 (1H, m, Glc-2H), 5.73 (1H, d, $J$=8.1 Hz, Glc-1H), 6.39 (1H, d, $J$=8.6 Hz, -CH=CHCOO-), 7.25 (1H, d, $J$=16.11 Hz, ph-H), 7.29 (1H, d, $J$=8.6 Hz, -CH=CHCOO-), 7.55 (1H, s, ph-H), 7.63 (1H, d, $J$=16.11 Hz, ph-H).

MS m/z (relative abundance %): 357 ([M+H]$^+$, 5), 356 (10), 355 (8), 311 (3), 275 (78), 183 (100), 179 (12), 91 (99), 89 (32), 59 (35).

**DPPH Radical-scavenging Effects** The test compounds (2 ml) were adjusted with ethanol solution to final concentration of 1 mM. Acetic acid buffer (0.1 mM) as added, and the mixture was warmed in tropical aquarium at 25°C. After 5 min, DPPH radical ethanol solution (1 ml, 0.2 mM) was added. After 30 min, absorbance was measured with a spectrophotometer (517 nm). The DPPH radical-scavenging rate of each sample was calculated and the 50% scavenging concentration based on the DPPH radical-scavenging rate was also calculated based on the following formula:

$$\text{Scavenging rate} = \frac{A_0 - A_t}{A_0} \times 100\%$$
DPPH radical-scavenging Rate (%) 
\[ = \left(1 - \frac{A - C}{B}\right) \times 100 \]

Where \( A \) is the absorbance of the sample when a blank was substituted for ethanol, \( B \) the absorbance of the sample when a color-contrast agent was substituted for ethanol in the DPPH radical-ethanol solution, and \( C \) the absorbance of the color-contrast agent alone.

**RESULTS AND DISCUSSION**

DPPH radicals are widely used for the preliminary screening of compounds capable of scavenging activated oxygen species since they are much more stable and easier to handle than oxygen free radicals. To increase the hydrophilic properties of the compounds and enhance their permeability, glycoside derivatives of \( 3, 4, \) and \( 5 \) were synthesized to give \( 3a, 4a, \) and \( 5a \), respectively. Details of the synthesis are shown in Fig. 1. The existence of \( \alpha \)- and \( \beta \)-anomers was analyzed using the relationship between C-1 and C-2 protons that form the \( O \)-glycoside. The C-2 proton is considered to be an axial bond in the structure of glucose and depending on whether the C-1 proton is synthesized in the axial or equatorial position, it is possible to determine the type of anomer (according to the vicinal coupling of the C-1 and C-2 protons). Based on \( ^1\text{H}-\)

NMR data, the vicinal coupling of the C-1 proton of \( 3a, 4a, \) and \( 5a \) was 8.1 Hz, confirming that all three compounds represent the \( \beta \)-anomer. The effects of scavenging DPPH radicals were also investigated on the compound that contains citrusin \( 1a \) and iso-citrusin \( 2a \). The test results of the scavenging ratio of DPPH radicals of each compound and 50% scavenging concentration \((SC_{50})\) are shown in Table 1. By employing the DPPH radical test, favorable scavenging ratios were found for compounds \( 1, 2, 2a, 3a, 4, \) and \( 5 \). In particular, compounds \( 2 \) and \( 3 \) had a scavenging ratios ranging from 82.7% to 100%, even when diluted to 0.05 mM. These results are similar to those obtained for \( \alpha \)-tocopherol \( 9 \), which was used as a control agent for radical scavenging. To quantify the ability of each compound to scavenge free radicals, \( SC_{50} \) values were determined. The accuracy of this method was satisfactory since the inclination of the reaction curve was greatest at the 50% reaction rate in each case. Compounds \( 2, 3, \) and \( 3a \) had \( SC_{50} \) values for scavenging DPPH radicals of 19 \( \mu \)M, 8 \( \mu \)M, and 29 \( \mu \)M, respectively. These values are equivalent to that determined for the \( \alpha \)-tocopherol control (Table 1).

The radical-scavenging activity was also examined in terms of the chemical structures including those of the functional radical and its orientation. Hydroxy
Table 1. Rate of Scavenging DPPH Radical and 50% DPPH Radical-scavenging Concentration (SC50) for Each under Test Compounds

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<th>Compound</th>
<th>4×10^{-1}</th>
<th>2×10^{-1}</th>
<th>1×10^{-1}</th>
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<sup>a</sup> 50% scavenging concentration (μM).

Fig. 2.

and methoxy groups in the 1,4- and 1,3-orientation are mainly involved in the scavenging of propenyl acid. This was observed for compounds 3 and 4 containing the hydroxyl-group in the 1,4-orientation. We also found that scavenging activity tended to decrease slightly for compounds 3a and 4a in which a β-glucose moiety was introduced. Furthermore, the development of scavenging activity was not found for compounds 5 and 5a in which the hydroxyl-group is in the 1,3-orientation. Based on these results, a benzene ring where the hydroxyl radical is in the 1,4-orientation allows the oxygen atom to share a positive charge, thereby causing stabilization through delocalization. However, if the hydroxyl radical is in the 1,3-orientation, the oxygen atom is unable to share the charge and this is thought to influence the ability to scavenge the DPPH radicals. The substitution radical of the 1,2- or 1,4-orientation generally donates an electron to the aromatic ring to activate it, either by the resonance effect or inductive effect. However, a substitution radical in the 1,3-orientation tends to inactivate the ring.

This tendency was also found in the scavenging tests of DPPH radicals against phenylpropanoids undertaken by the authors.

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