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

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Eugenol, isoeugenol, caffeic acid, ferulic acid, isoferulic 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 isoferulic acid (SC_{50}=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.[1,2] 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.[3,4] 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 tisse injury skin.[5,6] 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.[7—10] 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.

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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**, isoferulic 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 ^{1}H-NMR (400 MHz) and MS spectra.

Synthesis

Synthesis of Glycoside When a reaction was shown, a solution of 3,4-diacetylcinnamic acid (0.240 g, 1.0×10^{-3} mol) and 1,1,3,3-tetramethylurea (0.2

ml, 1.7×10^{-3} mol) in CH_2Cl_2 (dehydrated, 5 ml) was added, and the mixture was stirred with aceto-bromo-α-D-glucose (0.617 g, 1.5×10^{-3} mol) and stannous triflate (0.625 g, 1.5×10^{-3} mol) in CH_2Cl_2 (5 ml) at room temperature (20±2℃). After 18 h, aqueous $NaHCO_3$ solution (100 ml) was added the solution, which was then extracted twice with CH_2Cl_2 (100 ml). The extract was washed with saturated aqueous NaCl, dried over anhydrous Na_2SO_4, evaporated under reduced pressure, purified by column chromatography on silica gel with hexane-EtOAc (1 : 1) to afford 3,4-diacetylcinnamic acid tetraacetylglycoside, 0.601 g (1.2×10^{-3} mol, 60.4%). After deacetylation, a solution of 3,4-diacetylcinnamic acid tetraacetylglycoside 0.253 g (5×10^{-4} mol) in CH_3OH–THF (1 : 1, 10 ml) was placed in CH_3ONa 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_3OH (5 : 1) to afford 3,4-dihydroxycinnamic acid glucopyranoside **3a** 0.025 g (2.9×10^{-5} mol, 15.0%).

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

1a Yield: 94.1%, $[\alpha]_D^{20}$ −46.60°, m.p. 132—133.2℃.
^{1}H–NMR δCD_3OD (ppm): 3.31 (2H, d, J=6.5 Hz, H-7), 3.68 (1H, dd, J=4.8, 12.1, H-6′a), 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-9a), 5.05 (1H, dd, J=1.6, 17.4 Hz, H-9b), 5.95 (1H, dd, J=6.6 14.2 Hz, H-8), 6.72 (1H, dd, 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%, $[\alpha]_D^{20}$ −42.84°, m.p. 129—131.5℃.
^{1}H–NMR $\delta CDCl_3$ (ppm): 3.84 (3H, s, -OCH_3), 6.03 (1H, dt, J=14.5, 6.0 Hz, H_β), 6.20 (1H, dt, J=14.5, 2.8 Hz, H_α), 6.76 (1H, dd, J=8.6, 3.2 Hz, H_6), 6.82 (1H, d, J=2.6 Hz, H_2), 6.99 (1H, d, J=7.0 Hz, H_5)
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%, $[\alpha]_D^{20}$ −34.31°, m.p. 181—182℃.
^{1}H–NMR $\delta CDCl_3$ (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%, $[\alpha]_D^{20}$ −21.28°, m.p. 172—174℃.
^{1}H–NMR $\delta CDCl_3$ (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=16.1Hz, 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%, $[\alpha]_D^{20}$ −25.29°, m.p. 176—178℃.
^{1}H–NMR $\delta CDCl_3$ (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℃. 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 calculate based on the following formula:

$$\text{DPPH radical-scavenging Rate}(\%) = \left(1-\frac{A-C}{B}\right)\times 100$$

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 α- and β-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}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 β-anomer. The effects of scavenging DPPH radicals were also investigated on the compound that contains citrusin C **1a** and *iso*-citrusin C **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 α-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 μM, 8 μM, and 29 μM, respectively. These values are equivalent to that determined for the α-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

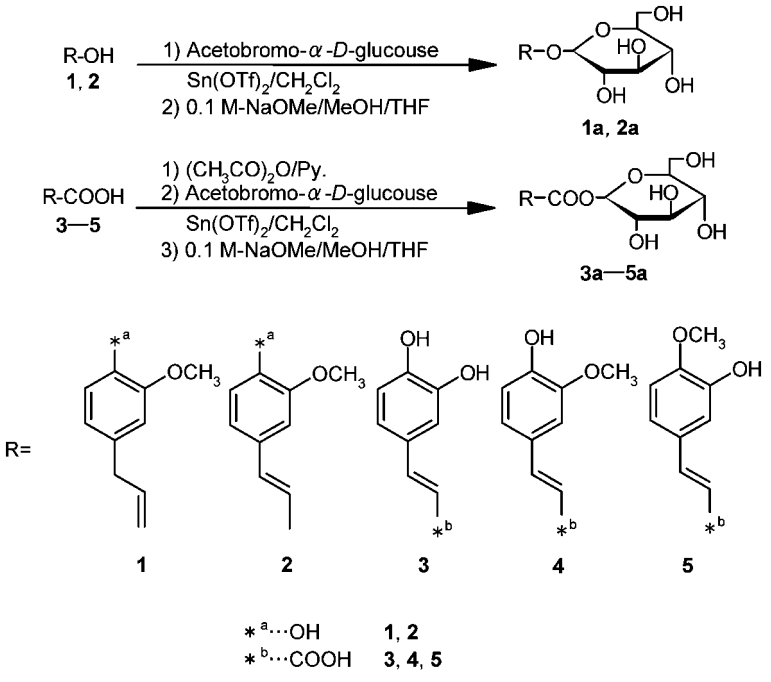
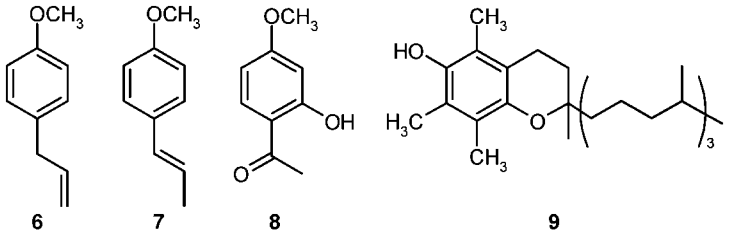


Fig. 1. Process for the Synthesis of **1a**—**5a**

Table 1. Rate of Scavenging DPPH Radical and 50% DPPH Radical–scavenging Concentration (SC_{50}) for Each under Test Compounds

Compound	Concentration (mM)								SC_{50}[a]
	4×10^{-1}	2×10^{-1}	1×10^{-1}	5×10^{-2}	2.5×10^{-2}	1.25×10^{-2}	6.25×10^{-3}	3.125×10^{-3}	
1	100.0	100.0	87.9	63.3	40.8	21.7	9.6	0.0	67
1a	0.0	0.0	0.0	0.0	0.0	—	—	—	>400
2	100.0	100.0	100.0	100.0	62.1	24.7	1.4	0.0	19
2a	97.6	84.2	63.3	41.7	22.9	—	—	—	62
3	—	—	—	82.7	79.8	62.1	42.4	24.5	8
3a	100.0	100.0	100.0	67.4	30.6	14.3	5.2	6.5	29
4	—	—	—	57.5	41.1	26.5	15.3	6.3	36
4a	76.9	62.5	41.7	23.8	12.7	—	—	—	134
5	37.8	25.3	15.3	11.3	1.3	—	—	—	677
5a	33.9	14.0	11.4	4.9	2.6	—	—	—	1002
6	0.0	0.0	0.0	0.0	0.0	—	—	—	>400
7	0.0	0.0	0.0	0.0	0.0	—	—	—	>400
8	0.0	0.0	0.0	0.0	0.0	—	—	—	>400
9	—	—	—	100.0	96.7	49.6	17.1	8.3	10

a) 50% scavenging concentration (μM).



6 7 8 9

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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