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In Vitro Free Radical and $ONOO^-$ Scavengers from *Sophora flavescens*

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Activity-guided fractionation of the CH_2Cl_2-soluble fraction of the roots of *Sophora flavescens* furnished five 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavengers: *trans*-hexadecyl ferulic acid (**1**), *cis*-octadecyl ferulic acid (**2**), *trans*-hexadecyl sinapic acid (**3**), (–)-4-hydroxy-3-methoxy-(6a*R*,11a*R*)-8,9-methylenedioxypterocarpan (**4**) and desmethylanhydroicaritin (**8**), along with nine known inactive compounds: (–)-maackiain (**5**), xanthohumol (**6**), formononetin (**7**), (2*S*)-2'-methoxykurarinone (**9**), (2*S*)-3β,7,4'-trihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (**10**), (2*S*)-7,4'-dihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (**11**), umbelliferone (**12**), kuraridin (**13**), and trifolirhizin (**14**). Compounds **1**-**4** and **8** exhibited DPPH free radical scavenging effects at IC_{50} values of 33.01 ± 0.20, 57.06 ± 0.16, 39.84 ± 0.36, 35.83 ± 0.47, and 18.11 ± 0.04 μM, respectively. L-Ascorbic acid, when used as a positive control, exhibited an IC_{50} value of 7.39 ± 0.01 μM. Compounds **1**-**4** and **8** also appeared to exert significant scavenging effects on authentic $ONOO^-$, with IC_{50} values of 5.76 ± 1.19, 15.06 ± 1.64, 8.17 ± 4.97, 1.95 ± 0.29, and 4.06 ± 2.41 μM, respectively. Penicillamine (IC_{50} = 2.36 ± 0.79 μM) was used as a positive control. In addition, compounds **2**, **4**, **6**, **8**, and **10** were isolated from this plant for the first time.

Key words: *Sophora flavescens*, 1,1-Diphenyl-2-picrylhydrazyl radical scavengers, $ONOO^-$ scavengers, *cis*-Octadecyl ferulic acid, (–)-4-Hydroxy-3-methoxy-(6a*R*,11a*R*)-8,9-methylenedioxypterocarpan

INTRODUCTION

Sophorae Radix, the dried roots of *Sophora flavescens* Aiton (Leguminosae) has long been used as a traditional Chinese medicine for the treatment of acute dysentery, gastrointestinal, hemorrhage, and eczema (Perry and Metzger, 1980; Huang, 1993). Several flavonoids (Kyogoku *et al.*, 1973; Woo *et al.*, 1998; Kuroyanagi *et al.*, 1999; Kang *et al.*, 2000; Ding *et al.*, 2005), alkaloids (Okuda *et al.*, 1965; Murakoshi *et al.*, 1982; Saito *et al.*, 1990), pterocarpans (Wu *et al.*, 1985), and saponins (Yoshikawa *et al.*, 1985; Ding *et al.*, 1992) have revealed from *S. flavescens*. As a part of our continuing studies to identify novel free radical scavengers from natural sources, the CH_2Cl_2 fraction from MeOH extract of *S. flavescens* was found to have the potent free radical scavenging activity by the DPPH test (IC_{50} = 37.01 μg/mL). In this paper, we report the isolation and structure identification of compounds **1**-**14**, which include five active compounds (**1**-**4** and **8**). Compounds **2**, **4**, **6**, **8**, and **10** were isolated from this plant for the first time.

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MATERIALS AND METHODS

Plant materials

The roots of *Sophora flavescens* Aiton (Leguminosae) were purchased from the herbal medicine co-operative association in Seoul Province, Korea. A voucher specimen (no. 20030320) has been deposited in the laboratory of Prof. J. S. Choi.

Chemicals

The 1,1-diphenyl-2-picrylhydrazyl (DPPH), L-ascorbic acid, and DL-2-amino-3-methylbutanoic acid (DL-penicillamine), *trans*-4-hydroxy-3-methoxycinnamic acid (*trans*-ferulic acid), *cis*-4-hydroxy-3-methoxycinnamic acid (*cis*-ferulic acid), *trans*-4-hydroxy-3,5-dimethoxycinnamic acid (*trans*-

sinapic acid), kaempferol, were purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.). The high quality 2',7'-dichlorofluorescin diacetate (DCFH-DA), dihydrorhodamine 123 (DHR 123) and $ONOO^-$ were purchased from Molecular Probes (Eugene, Oregon, U.S.A.) and Cayman (Ann Arbor, MI, U.S.A.), respectively.

General experimental procedures

UV spectra were recorded on a Varian Carry UV-visible spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were determined with a JEOL JNM ECP-400 spectrometer, using $CDCl_3$ and DMSO-d_6. HMQC and HMBC spectra were recorded using pulsed-field gradients. EI-MS data were recorded on a JEOL JMS-700 spectrometer. Optical rotation was obtained with a Perkin-Elmer Polarimeter 341. CD was recorded with a JASCO-715 spectro-polarimeter. Column chromatography was carried out using silica (Si) gel 60 (70-230 mesh, Merck, Germany), RP-18 gel (40-63 mm, Merck, Germany), and Sephadex LH-20 (25-100 μ, Sigma, St. Louis, MO). TLC was carried out on a precoated Merck Kieselgel 60 F_{254} plate (20×20 cm, 0.25 mm) and a RP-18 F_{254s} plate (5×10 cm, Merck, Germany), and 50% H_2SO_4 was used as a spray reagent. All solvents for column chromatography were of reagent grade, and were acquired from commercial sources.

Extraction and isolation

The roots (5 kg) of *Sophora flavescens* were refluxed with MeOH for three hours (3×10 L). The total filtrate was concentrated to dryness in *vacuo* at 40°C in order to render the MeOH extract (1.1 kg) and this extract was suspended in distilled water and sequentially partitioned with CH_2Cl_2 (114 g), EtOAc (124 g), *n*-BuOH (305 g), and H_2O (524 g) in sequence. The CH_2Cl_2 fraction (70 g) was initially chromatographed over a Si gel column using CH_2Cl_2 - MeOH under gradient conditions (CH_2Cl_2 → CH_2Cl_2 : MeOH = 80:1 → 1:1, MeOH, gradient) in order to yield 18 subfractions (Fr.1 - Fr.18). A portion of fraction 5 (770 mg) was purified by Si gel with *n*-hexane - EtOAc (12:1 to 1:1, EtOAc, gradient), yielding compounds **1** (25 mg) and **2** (10 mg). Fraction 9 (8.77 g) was subjected to column chromatography over a Si gel column with CH_2Cl_2 - MeOH (100:1 to 1:1, MeOH, gradient) to yield compounds **3** (30 mg), **4** (20 mg), **5** (140 mg), and **6** (15 mg). Fraction 10 (9.15 g) was further purified, resulting in the isolation of compound **7** (30 mg). Fraction 11 (6.6 g) was purified by Si gel with CH_2Cl_2 - MeOH (40:1 to 1:1) to produce compounds **8** (20 mg), **9** (50 mg), **10** (35 mg), and **11** (50 mg). Fraction 14 (4.25 g) was chromatographed on a Sephadex LH-20 and RP-18 gel column with H_2O - MeOH (gradient) to yield compounds **12** (100 mg) and **13** (20 mg), respectively. Fraction 15 (1.56 g) was chromatographed on a Si gel column using CH_2Cl_2 - MeOH (10:1 to 1:1, MeOH, gradient) to yield compound **14** (200 mg).

trans-Hexadecyl ferulic acid (1)

Colorless needles; EIMS (*m/z*, %): 418 (M^+, 100), 194 [$(M-C_{16}H_{32})^+$, 75], 177 [$(M-C_{16}H_{32}O-H)^+$, 27], 150 [$(M-C_{17}H_{32}O_2)^+$, 19], 137 (24), 117 (8), 89 (9); ^{1}H-NMR (400 MHz, $CDCl_3$) δ : 7.61 (1H, d, *J*=16.1 Hz, H-7), 7.08 (1H, dd, *J*=1.9, 8.1 Hz, H-6), 7.04 (1H, d, *J*=1.9 Hz, H-2), 6.92 (1H, d, *J*=8.1 Hz, H-5), 6.29 (1H, d, *J*=16.1 Hz, H-8), 4.19 (2H, t, *J*=6.7 Hz, H-1'), 3.93 (3H, s, OCH_3), 1.70 (2H, m, H-2'), 1.25 (26H, s, H-3'~15'), 0.88 (3H, t, *J*=6.5 Hz, H-16'); ^{13}C-NMR (100 MHz, $CDCl_3$) δ : 167.4 (C-9), 147.9 (C-4), 146.7 (C-3), 144.1 (C-7), 127.0 (C-1), 123.0 (C-6), 115.7 (C-5), 114.7 (C-8), 109.3 (C-2), 64.4 (C-1'), 55.9 (OMe), 31.9 (C-14'), 29.7, 29.6, 29.5, 29.4, 29.3, 28.8 (C-5'~13'), 25.9 (C-3'), 22.7 (C-15'), 14.1 (C-16').

cis-Octadecyl ferulic acid (2)

EIMS (*m/z*, %): 446 (M^+), 194 [$(M-C_{17}H_{32}O)^+$, 100], 177 [$(M-C_{17}H_{32}O_2-H)^+$, 73]; ^{1}H-NMR (400 MHz, $CDCl_3$) δ : 7.77 (1H, d, *J*=1.9 Hz, H-2), 6.88 (1H, d, *J*=8.1 Hz, H-5), 7.10 (1H, dd, *J*=1.9, 8.1 Hz, H-6), 6.79 (1H, d, *J*=12.9 Hz, H-7), 5.82 (1H, d, *J*=12.9 Hz, H-8), 4.12 (2H, t, *J*=6.7 Hz, H-1'), 3.93 (3H, s, OCH_3), 1.68 (2H, m, H-2'), 1.25 (30H, s, H-3'~17'), 0.88 (3H, t, *J*=6.5 Hz, H-18'); ^{13}C-NMR (100 MHz, $CDCl_3$) δ : 166.6 (C-9), 147.0 (C-4), 145.9 (C-3), 143.6 (C-7), 127.3 (C-1), 125.6 (C-6), 116.9 (C-8), 113.8 (C-5), 112.8 (C-2), 64.4 (C-1'), 55.9 (OMe), 31.9 (C-3'), 29.7, 29.6, 29.5, 29.4 (C-5'~15'), 22.7 (C-17'), 14.1 (C-18').

trans-Hexadecyl sinapic acid (3)

EIMS (*m/z*, %): 448 (M^+), 224 [M^+-$(CH_2)_{15}CH_3$+H, 88], 207 (224-OH, 43), 180 (224-COOH+H, 30), 167 (224-CHCOOH+H, 39); ^{1}H-NMR (400 MHz, DMSO-d_6) δ : 7.54 (1H, d, *J*=15.9 Hz, H-7), 7.02 (2H, s, H-2, 6), 6.52 (1H, d, *J*=15.9 Hz, H-8), 4.11 (2H, t, *J*=6.7 Hz, H-1'), 3.77 (6H, s, OCH_3×2), 1.63 (2H, m, H-2'), 1.22 (26H, s, H-3'~15'), 0.88 (3H, t, *J*=6.5 Hz, H-16'); ^{13}C-NMR (100 MHz, DMSO-d_6) δ : 166.6 (C-9), 148.0 (C-3, 5), 138.4 (C-7), 124.3 (C-1), 114.9 (C-8), 106.2 (C-2, 6), 63.7 (C-1'), 56.1 (OMe), 31.2 (C-14'), 28.9, 28.8, 28.7, 28.6 (C-5'~13'), 25.4 (C-3'), 22.1 (C-15'), 13.9 (C-16').

(–)-4-Hydroxy-3-methoxy-(6a*R*,11a*R*)-8,9-methylenedioxypterocarpan (4)

Colorless needles; m.p. 172-173°C; EIMS (*m/s*, %): 314 (M^+, 100); $[\alpha]_{20}^{D}$: -14.12° (c=0.04, MeOH); UV λ_{max} (MeOH): 241 (sh, log ε 3.69), 312 (3.59) nm; + NaOMe 309 (3.66); + NaOAc 311 (3.66); + NaOAc + H_3BO_3 241 (3.72), 313 (3.59); + $AlCl_3$ 240 (3.70), 311 (3.59); + $AlCl_3$ + HCl 240 (3.67), 312 (3.58) nm; ^{1}H-NMR (400 MHz, $CDCl_3$) δ : 7.04

(1H, d, *J*=8.6 Hz, H-1), 6.73 (1H, s, H-7), 6.67 (1H, d, *J*=8.6 Hz, H-2), 6.44 (1H, s, H-10), 5.91 (2H, each d, *J*=12.9 Hz, -OCH_2O-), 5.52 (1H, d, *J*=6.9 Hz, H-11a), 4.34 (1H, dd, *J*=5.0, 10.9 Hz, H-6eq), 3.91 (3H, s, OCH_3), 3.70 (1H, t, *J*=10.9 Hz, H-6ax), 3.52 (1H, m, H-6a); ^{13}C-NMR (100 MHz, $CDCl_3$) δ : 154.2 (C-10a), 148.2 (C-9), 147.3 (C-3), 143.3 (C-4a), 141.8 (C-8), 134.0 (C-4), 121.0 (C-1), 117.7 (C-6b), 113.9 (C-11b), 105.4 (C-2), 104.8 (C-7), 101.3 (-OCH_2O-), 93.8 (C-10), 78.4 (C-11a), 66.9 (C-6), 56.3 (OMe), 40.3 (C-6a).

Xanthohumol (6)

^{1}H-NMR (400 MHz, DMSO-d_6) δ : 7.76 (1H, d, *J*=15.3 Hz, H-2), 7.66 (1H, d, *J*=15.3 Hz, H-3), 7.57 (2H, d, *J*=8.6 Hz, H-2', 6'), 6.84 (2H, d, *J*=8.6 Hz, H-3', 5'), 6.07 (1H, s, H-6), 5.14 (1H, br t, *J*=6.9 Hz, H-2"), 3.86 (3H, s, OCH_3), 3.13 (2H, d, *J*=6.9 Hz, H-1"), 1.70 (3H, br s, H-4"), 1.60 (3H, br s, H-5"); ^{13}C-NMR (100 MHz, DMSO-d_6) δ : 191.4 (C-4), 164.6 (C-5), 162.9 (C-7), 160.5 (C-9), 159.9 (C-4'), 142.3 (C-2), 130.4 (C-2', 6'), 129.8 (C-3"), 126.0 (C-1'), 123.8 (C-3), 123.1 (C-2"), 115.9 (C-3', 5'), 107.3 (C-8), 104.4 (C-10), 91.1 (C-6), 55.7 (OMe), 25.5 (C-4"), 21.0 (C-1"), 17.7 (C-5").

Desmethylanhydroicaritin (8)

^{1}H-NMR (400 MHz, DMSO-d_6) δ : 12.40 (1H, s, 5-OH), 10.73 (1H, s, 7-OH), 10.12 (1H, s, 4'-OH), 9.36 (1H, s, 3-OH), 8.03 (2H, d, *J*=8.9 Hz, H-2', 6'), 6.93 (2H, d, *J*=8.9 Hz, H- 3', 5'), 6.29 (1H, s, H-6), 5.17 (1H, t, *J*=6.8 Hz, H-2"), 3.42 (2H, d, *J*=6.8 Hz, H-1"), 1.74 (3H, br s, H-4"), 1.62 (3H, br s, H-5"); ^{13}C-NMR (100 MHz, DMSO-d_6) δ : 176.1 (C-4), 161.1 (C-7), 159.1 (C-4'), 158.3 (C-5), 153.4 (C-9), 146.7 (C-2), 135.5 (C-3), 130.9 (C-3"), 129.3 (C-2', 6'), 122.5 (C-2"), 121.9 (C-1'), 115.4 (C-3', 5'), 105.6 (C-8), 102.9 (C-10), 97.8 (C-6), 25.4 (C-5"), 21.2 (C-1"), 17.8 (C-4").

(2*S*)-3β,7,4'-Trihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (10)

Yellow amorphous powder; $[\alpha]_{20}^{D}$: -0.71° (c=0.02, MeOH); UV (MeOH) λ_{max} (log ε) 289.3 (4.03) nm, 325.9 (3.57) nm; CD (c 1.2×10^{-4}, MeOH) $[\theta]_{335}$ + 3.46, $[\theta]_{289}$ – 16.32; ^{1}H-NMR (400 MHz, DMSO-d_6) δ : 7.29 (2H, d, *J*=8.6 Hz, H-2', 6'), 6.77 (2H, d, *J*=8.6 Hz, H-3', 5'), 6.16 (1H, s, H-6), 5.08 (1H, br t, *J*=6.8 Hz, H-2"), 4.90 (1H, d, *J*=11.3 Hz, H-2), 4.25 (1H, d, *J*=11.3 Hz, H-3), 3.70 (3H, s, OCH_3), 3.06 (2H, d, *J*=6.8 Hz, H-1"), 1.53 (3H, br s, H-4"), 1.50 (3H, br s, H-5"); ^{13}C-NMR (100 MHz, DMSO-d_6) δ : 190.3 (C-4), 161.1 (C-7), 160.9 (C-9), 160.8 (C-4'), 159.5 (C-5), 130.0 (C-3"), 129.0 (C-2', 6'), 128.1 (C-1'), 122.6 (C-2"), 114.8 (C-3', 5'), 107.3 (C-8), 102.4 (C-10), 92.8 (C-6), 82.2 (C-2), 72.5 (C-3), 55.3 (OMe), 25.5 (C-4"), 21.3 (C-1"), 17.5 (C-5").

Measurement of the DPPH radical scavenging activity

The DPPH radical scavenging effect was evaluated as previously described by Blois (1958) with minor modifications. A methanolic sample solution of 160 μL at several concentrations and 40 μL of the DPPH methanolic solution (1.5×10^{-4} M) were added to a 96-well microplate, in a total volume of 200 μL. After letting the reaction mixture stand at room temperature for 30 min, its absorbance was determined at 520 nm, in a microplate reader (VERSA max, Molecular device, CA, U.S.A.). The scavenging effects of each sample was expressed in terms of IC_{50} (μg/mL or μM required to inhibit DPPH radical formation by 50%) and calculated from the log-dose inhibition curve.

Measurement of the $ONOO^-$ scavenging activity

The $ONOO^-$ scavenging activity was measured by monitoring the oxidation of DHR 123 by modifying the method of Kooy *et al.*, (1994). The DHR 123 (5 mM) in dimethylformamide, purged with nitrogen, was stored at -80°C and used as a stock solution. This solution was then placed in ice and was not exposed to light prior to the study. The buffer used consisted of 90 mM sodium chloride, 50 mM sodium phosphate, 5 mM potassium chloride at pH 7.4 and 100 μM diethylenetriaminepentaacetic acid (DTPA), each of which was prepared with high quality deionized water and purged with nitrogen. The final concentration of the DHR 123 was 5 μM. The background and final fluorescent intensities were measured 5 min after treatment, both with and without the addition of authentic $ONOO^-$, DHR 123 was oxidized rapidly by authentic $ONOO^-$, and its final fluorescent intensity remained unchanged over time. The fluorescent intensity of oxidized the DHR 123 was measured with a microplate fluorescence reader FLx 800 (Bio-Tek Instruments Inc.) at the excitation and emission wavelengths of 480 nm and 530 nm, respectively. Results were expressed as the mean ± standard error (*n* = 3) for the final fluorescence intensity minus the background fluorescence. The effects were expressed as the percent inhibition of oxidation of DHR 123.

Statistical analysis

All values were expressed as the mean ± standard error of three or five replicate experiments.

RESULTS AND DISCUSSION

Free radicals can be generated from the metabolic pathways inherent in normal and pathological cells. However, they can be produced in much greater abundance upon exposure to external factors, including the addition

of foreign materials or exposure to UV radiation. Free radicals and reactive oxygen or nitrogen species, including the hydroxyl radical and peroxynitrite, have been confidently implicated in the etiology of a vast of human degenerative diseases (Pincemail, 1995).

1,1-Diphenyl-2-picrylhydrazyl (DPPH) is a free radical compound which has been widely used in assessments of the free radical scavenging ability of a variety chemicals (Beckman *et al.*, 1990). While screening for free radical scavenging activity in a series of medicinal plants, the CH_2Cl_2-soluble fraction of the roots of *Sophora flavescens* Aiton (Leguminosae) was discovered to display some promising activities (IC_{50} = 37.01 μg/mL). The search for the compounds responsible for radical scavenging activity in the CH_2Cl_2-soluble fractions was carried out by subjecting various fractions obtained from column chromatography to the DPPH-*in vitro* model. The CH_2Cl_2-soluble fraction was successive-column-chromatographed over silica gel, Sephadex LH-20 and RP-18, which furnished five free radical scavengers: *trans*-hexadecyl ferulic acid (**1**, Kim *et al.*, 2001), *cis*-octadecyl ferulic acid (**2**, Baldé *et al.*, 1991), *trans*-hexadecyl sinapic acid (**3**, Zhang *et al.*, 2000), (–)-4-hydroxy-3-methoxy-(6a*R*,11a*R*)-8,9-methylenedioxypterocarpan (**4**, Kim *et al.*, 2002), and desmethylanhydroicaritin (**8**, Mizuno *et al.*, 1988), along with nine known inactive compounds, (–)-maackiain (**5**, Park *et al.*, 2003), xanthohumol (**6**, Tabata *et al.*, 1997), formononetin (**7**, Ryu *et al.*, 1997), (2*S*)-2'-methoxykurarinone (**9**, Kang *et al.*, 2000), (2*S*)-3β,7,4'-trihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (**10**, Jakupovic *et al.*, 1988), (2*S*)-

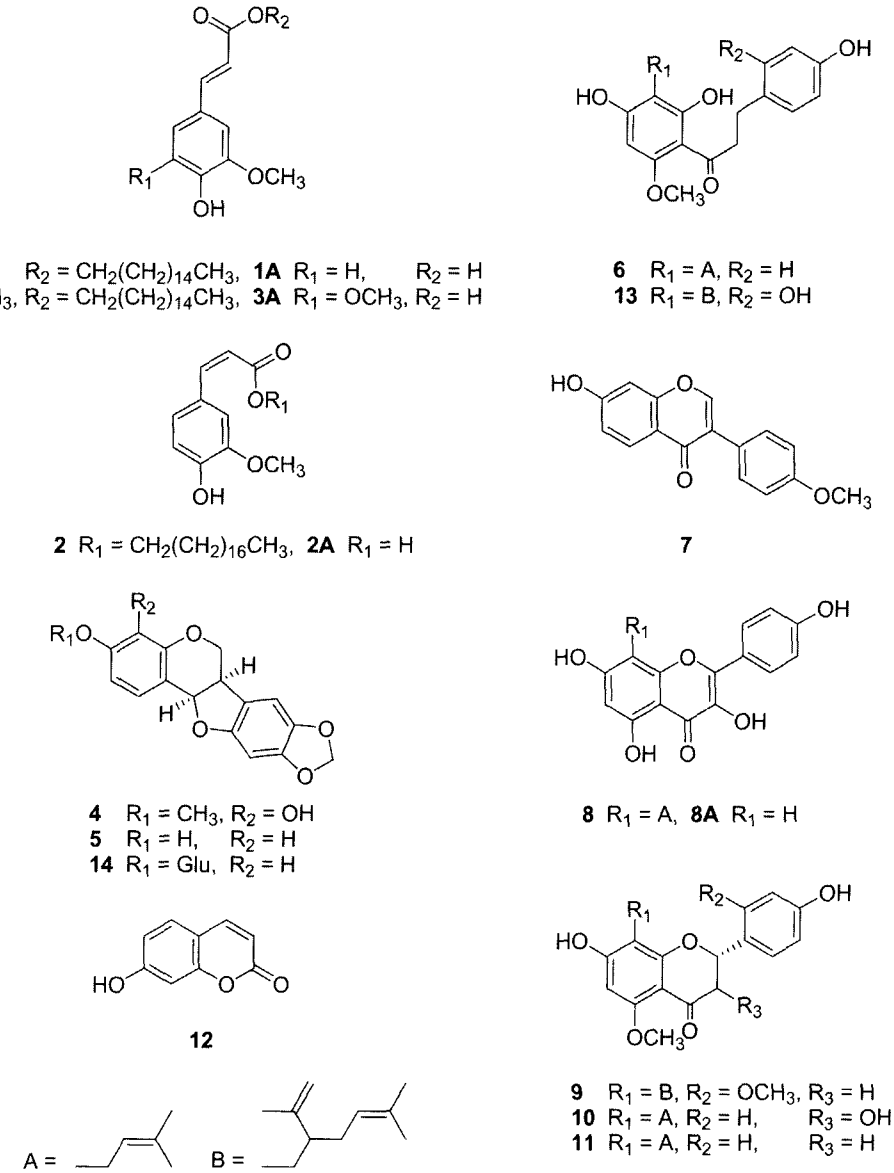

Fig. 1. Chemical structures of compounds **1**-**14**

7,4'-dihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone (**11**, Komatsu *et al.*, 1970), umbelliferone (**12**, Jung *et al.*, 1994), kuraridin (**13**), and trifolirhizin (**14**, Ryu *et al.*, 1997), respectively. The structural identification of these compounds was carried out by an analysis of 1D (^{1}H- and ^{13}C-NMR) and 2D NMR (HMQC and HMBC) spectral data, and by comparisons with published spectral data. Our results revealed that compounds **2**, **4**, **6**, **8**, and **10** had never been isolated from this plant. Although Baldé *et al.* (1991) had obtained a mixture (2:1) of octadecyl *trans*- and *cis*-ferulates from a hexane extract of *Pavetta owariensis*, the pure form of octadecyl *cis*-ferulic acid (**2**) has only been separated from the CH_2Cl_2 fraction of *S. flavescens*. To the best of our knowledge, the ^{13}C-NMR spectral data of **2** and **4** have also never been previously reported. The chemical structures of compounds **1-14** are shown in Fig. 1. The scavenging effects of isolated compounds **1-4** and **8** are shown in Table I and Fig. 2. Compounds **1-4** and **8** all exhibited DPPH radical scavenging effects, with IC_{50} values of 33.01 ± 0.20, 57.06 ± 0.16, 39.84 ± 0.36, 35.83 ± 0.47, and 18.11 ± 0.04 μM, respectively. L-Ascorbic acid exhibited an IC_{50} value of 7.39 ± 0.01 μM.

Peroxynitrite ($ONOO^-$), formed as a result of the reaction of superoxide and nitric oxide, is a cytotoxic species which is able to oxidize several cellular components, including proteins, lipids, and DNA. It has been implicated in several diseases, such as Alzheimer's disease, rheumatoid arthritis, cancer, and atherosclerosis (Squadrito and Pryor, 1998). Moreover, the necessity for a strong $ONOO^-$ scavenger is clear, due to the absence of any enzyme which protects against the damage caused by $ONOO^-$. Thus, it is obviously desirable to isolate substances from natural products which exert protective effects against $ONOO^-$ derived diseases. Therefore, compounds **1-4** and **8** were evaluated with regard to their $ONOO^-$ scavenging properties. Although the relative $ONOO^-$ scavenging activities of these compounds did not precisely coincide with their relative free radical scavenging activities, compounds **1-4** and **8** did exhibit $ONOO^-$ scavenging activities at IC_{50} values of 5.76 ± 1.19, 15.06 ± 1.64, 8.17 ± 4.97, 1.95 ± 0.29, and 4.06 ± 2.41 μM, respectively. Penicillamine was found to exhibit an IC_{50} value of 2.36 ± 0.79 μM. However, the other compounds (**5-7**, **9-14**) showed no activity at the highest concentration (100 μM) in both free radical and $ONOO^-$ scavenging tests. As shown in Table I, the activity of phenolic acid with the *cis*-form aliphatic long chain was less robust than that the activity of the *trans*-form phenolic acid (**1** *vs* **2**). Derivatives which harbored an alkyl long chain exhibited weak scavenging effects on both free radicals and $ONOO^-$, relative to the available corresponding parent compounds (**1** *vs* **1A**, **3** *vs* **3A**, and **8** *vs* **8A**).

Table I. Scavenging effects of compounds **1-4** and **8**, and related parent compounds (**1A**, **3A**, and **8A**) on DPPH and $ONOO^-$

Compounds	IC_{50} (μM)	
	DPPH[a]	$ONOO^-$[b]
1	33.01 ± 0.20	5.76 ± 1.19
2	57.06 ± 0.16	15.06 ± 1.64
3	39.84 ± 0.36	8.17 ± 4.97
4	35.83 ± 0.47	1.95 ± 0.29
8	18.11 ± 0.04	4.06 ± 2.41
1A (*trans* ferulic acid)	9.79 ± 0.07	0.35 ± 0.22
3A (*trans* sinapic acid)	3.28 ± 0.05	0.47 ± 0.12
8A (kaempferol)	6.33 ± 0.01	1.12 ± 0.26
Penicillamine		2.36 ± 0.79
L-Ascorbic acid	7.39 ± 0.01	

[a] DPPH is the free radical scavenging activity (IC_{50}: μM).
[b] $ONOO^-$ is the inhibitory activity of peroxynitrite (IC_{50}: μM).

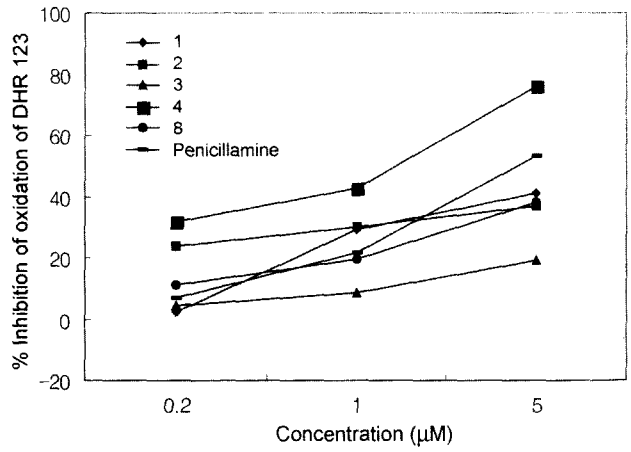

Fig. 2. Concentration-dependent scavenging of compounds **1-4**, **8**, and penicillamine on authentic $ONOO^-$

The scavenging effects of active compounds **1-4** and **8** on both DPPH radicals and $ONOO^-$ are believed to be attributable to their hydrogen-donating ability (Hatano *et al.*, 1989). These results suggest that flavonoids, hydroxycinnamates, and related phenolic acids serve as potent free radical scavengers, by virtue of their hydrogen-donation properties (Rice-Evans *et al.*, 1996). Thus, the free radical and $ONOO^-$ scavenging activities exhibited by these compounds may be principally related to their phenolic hydroxyl groups, and this may play an important role in the reported actions of *S. flavescens*.

The present work resulted in the partial isolation of free radical scavengers from *S. flavescens*. Further investigation of the free radical-scavenging activity of these natural compounds may provide novel techniques for the prevention of a variety of radical-mediated injures in pathological contexts *in vivo*. Further research into free radical scavengers is, in fact, currently in progress.

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