Antioxidative Phenolic Compounds from the Roots of *Rhodiola sachalinensis* A. Bor

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(Received January 4, 2000)

The acetone extract of the roots of *Rhodiola sachalinensis* has furnished six phenolic compounds which exhibited significant scavenging effects against DPPH free radical. The structures of these compounds were identified and determined as gallic acid (**1**), (-)-epigallocatechin 3-O-gallate (**2**), kaempferol (**3**), kaempferol 7-O-α-L-rhamnopyranoside (**4**), herbacetin 7-O-α-L-rhamnopyranoside, (**5**) and rhodiolinin (**6**) by physico-chemical and spectral evidences.

Key words: *Rhodiola sachalinensis*, Crassulaceae, Phenolic compound, Flavonoid, Flavono-lignan, Rhodiolinin, Antioxidant, DPPH radical

INTRODUCTION

The roots of *Rhodiola sachalinensis* A. Bor (Crassulaceae) have been used in northeastern Asia as a traditional medicine for the remedies of asthma and hemorrhage and also known to have tonifying and anti-aging activities. This plant grows at high rocky mountain area of Korea, China, and Japan (Ohwi, 1984). The plant of *Rhodiola* species has been known to produce various compounds such as flavonoids, monoterpene glycosides, cyanoglycosides, phenethyl glycosides and aliphatic glycosides, some of which showed antiallergic activities. (Yoshikawa *et al.*, 1997). Recently, several papers reported that extract of this plant showed hepatoprotective activities. (Udintsev *et al.*, 1992), inhibitory activities of ethanol absorption (Kim and Park, 1997) and antioxidative effect (Ryu, 1998). Among isolated compounds, flavonoids were occurred as main components (Kurkin *et al.*, 1982; Zapesochnaya and Kurkin, 1983) from *R. sachalinensis*. However, there are only a few reports about bioactive components of this plant. In our search for antioxidative components from medicinal plants, the acetone extract of the roots of *R. sachalinensis* was found to exhibit significant antioxidative activity, based on the scavenging activity of the stable DPPH free radical. The active compounds were isolated from the acetone extract of the roots of *R. sachalinensis* and the structures of these compounds were determined by analysis of EI- and negative FAB-MS as well as ^{1}H- and ^{13}C -NMR spectra.

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METERIALS AND METHODS

General

^{1}H and ^{13}C NMR spetra were recorded on a Varian Unity at 300, 500 MHz (^{1}H NMR) and 75,125 MHz (^{13}C NMR), respectively. Chemical shifts are given in (ppm) scale with TMS as internal standard. Negative FAB-MS was measured on a VG70-VSEQ at 35 KeV with glycerol matrix and EI MS on a GC-MS/MS-DSs TSQ700 at 30 eV. Column chromatography was carried out on Sephadex LH-20 (25~100 μm, Pharmacia), MCI-gel CHP-20P (75~150 μm, Mitsubishi) and YMC-gel ODS-A (70-230 and 400-500 mesh, YMC Co.). TLC was conducted on a precoated silica gel 60 F_{254} plate (Merck). Spots were detected under UV and by spraying with $FeCl_3$ and dil. H_2SO_4, followed by heating. Ascorbic acid, BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) were obtained from Sigma Chemical Co.

Plant material

The roots (3 kg) of *R. sachalinensis* were collected from Baik-du Mt. in July 1998. The voucher specimens (HKC 9871) are deposited at the herbarium in College of Pharmacy,

Chung-Ang University (Korea).

Extraction and isolation

Dried and pulverized roots were extracted with 80% aq. Me_2CO at room temp. After removal of Me_2CO *in vacuo,* the aq. soln was filtered. The filtrate was concentrated and then applied to a column of Sephadex LH 20. Elution with H_2O containing increasing proportions of MeOH afforded 3 fractions, fr I. (50 g), fr II. (30 g) and fr III. (40 g). Repeated column chromatography of fraction on Sephadex LH-20 with 60% MeOH yielded gallic acid (**1**, 900 mg). Column chromatography of fraction II over MCI-gel CHP-20P and YMC-gel ODS-A with H_2O-MeOH gradient yielded kaempferol 7-O-α-L-rhamnopyranoside (**4**, 20 mg) and herbacetin 7-O-α-L-rhamnopyranoside (**5**, 92 mg) and rhodiolinin(**6**, 80 mg). Column chromatography of fraction III over YMC gel ODS-A with 60% MeOH yielded kaempferol (**3**, 73 mg) and (-)-epigallocatechin 3-O-gallate (**2**, 97 mg). Each solvent fraction was prepared through common method.

Gallic acid (**1**): white amorphous powder. IR ν_{max}^{KBr} cm^{-1} : 3282 (Phenolic OH), 1648 (COOH), 1611, 1541 (Aromatic C=C). EI MS: *m/z* 170 $[M]^+$, ^{1}H-NMR (300 MHz, Acetone-d_6+D_2O): 7.15 (2H in total, s, galloyl H). ^{13}C NMR (75 MHz, Acetone-d_6+D_2O): δ 169.0 (COO), 146.0 (C-3, 5), 138.8 (C-4), 121.8 (C-1), 108.7 (C-2, 6).

(-)-Epigallocatechin 3-O-gallate (**2**): brown amorphous powder. $[\alpha]_D^{20}$: -120.0°(Acetone, *c*=0.8). IR ν_{max}^{KBr} cm^{-1}: 3367 (OH), 1612, 1536, 1451 (Aromatic C=C). EI MS: *m/z* 458 $[M]^+$. ^{1}H-NMR (300 MHz, Acetone-d_6+D_2O): 7.05 (2H in total, s, galloyl H), 6.67 (2H, s, H-2',6'), 6.06 (1H, d, *J*=2.0 Hz, H-8), 6.03 (1H, d, *J*=2.0 Hz H-6), 5.55 (1H, m, H-3), 5.04 (1H, s, H-2), 3.10-2.80 (2H in total, m, H-4). ^{13}C-NMR (75 MHz, Acetone-d_6+D_2O): δ 166.8 (galloyl CO), 157.8 (C-9), 157.6 (C-7), 157.0 (C-5), 146.3 (galloyl C-3, 5), 146.0 (C-3', 5'), 139.0 (galloyl C-4), 133.1 (C-4'), 130.7 (C-1'), 121.5 (galloyl C-1), 110.0 (galloyl C-2, 6), 106.6 (C-2', 6'), 98.7 (C-10), 96.4 (C-6), 95.5 (C-8), 78.0 (C-2), 69.8 (C-3), 26.5 (C-4).

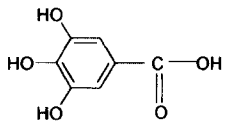
Kaempferol (**3**): yellow amorphous powder. IR ν_{max}^{KBr} cm^{-1}: 3316 (OH), 1656 (C=O), 1617, 1509 (Aromatic C=C). EI MS: *m/z* 286 $[M]^+$. ^{1}H-NMR (300MHz, DMSO-d_6): 12.46 (OH-5), 8.02 (2H in total, d, *J*=8.7 Hz, H-2',6'), 6.91(2H in total, d, *J*=8.7 Hz, H-3',5'), 6.42(1H, d, *J*=2.1 Hz, H-8), 6.17(1H, d, *J*=2.1 Hz, H-6). ^{13}C-NMR (75MHz, DMSO-d_6): δ 176.1 (C-4), 164.1 (C-7), 160.9 (C-5), 159.4 (C-4'), 156.4 (C-9), 147.0 (C-2), 135.8 (C-3), 129.6 (C-2',6'), 121.8 (C-1'), 115.6 (C-3',5'), 103.1 (C-10), 98.3 (C-6), 93.6 (C-8).

Kaempferol-7-O-α-L-rhamnopyranoside (**4**): yellow amorphous powder. $[\alpha]_D^{20}$: -156.0(Acetone, c=0.5). IR ν_{max}^{KBr} cm^{-1} : 3410 (OH), 1653 (C=O), 1594, 1497 (Aromatic C=C), Negative FAB MS: *m/z* 433 $[M-H]^-$. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 12.05 (1H, s, OH-5), 8.10 (2H in total, d, *J*= 8.7 Hz, H-2', 6'), 6.94 (2H in total, d, *J*=8.7 Hz, H-3', 5'), 6.85 (1H, d, *J*=2.0 Hz, H-8), 6.44 (1H, d, *J*= 2.0Hz, H-6), 5.56 (1H, s, H-1''), 1.14 (3H in total, d, *J*=6.3 Hz, rha-CH_3), ^{13}C-NMR (75MHz, DMSO-d_6): δ 176.3 (C-4), 161.6 (C-7), 160.6 (C-5), 159.6 (C-4'), 155.9 (C-9), 147.6 (C-2), 136.2 (C-3), 129.8 (C-2', 6'), 121.7 (C-1'), 115.6 (C-3', 5'), 104.8 (C-10), 98.9 (C-6), 98.4 (C-1''), 94.4 (C-8), 71.6 (C-4''), 70.3 (C-3''), 70.1 (C-2''), 69.9 (C-5''), 17.9 (C-6'').

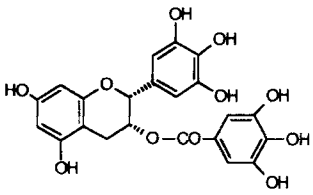
herbacetin 7-O-α-L-rhamnopyranoside (**5**): green amorphous powder. $[\alpha]_D^{20}$: -80.2(Acetone, c=0.7). IR ν_{max}^{KBr} cm^{-1}: 3385 (OH), 1656 (C=O), 1608, 1563 (Aromatic C =C). Negative FAB MS: *m/z* 449 $[M-H]^-$. ^{1}H NMR (300 MHz, DMSO-d_6): 11.89 (1H, s, OH-5), 8.16 (2H in total, d, *J*=9.0 Hz, H-2', 6'), 6.97 (2H in total, d, *J*=9.0 Hz, H-3', 5'), 6.61 (1H, s, H-6), 5.50 (1H, d, *J*=1.5 Hz, H-1''), 1.13 (3H in total, d, *J*=6.3Hz, CH_3). ^{13}C-NMR (75MHz, DMSO-d_6): δ 176.6 (C-4), 159.6 (C-4'), 151.8 (C-5), 150.3 (C-7), 147.6 (C-2), 144.7 (C-9), 136.0 (C-3), 130.0 (C-2', 6'), 127.2 (C-8), 122.0 (C-1'), 115.6 (C-3', 5'), 104.7 (C-10), 98.5 (C-1''), 98.4 (C-6), 71.8 (C-4''), 70.1 (C-3''), 70.0 (C-2'', 5''), 17.9 (C-6'').

Rhodiolinin (**6**): green amorphous powder. $[\alpha]_D^{20}$: -59.0° (Acetone, *c*=0.6). IR ν_{max}^{KBr} cm^{-1} : 3308 (OH), 1654 (C=O), 1601, 1569 (Aromatic C=C). Negative FAB MS: *m/z* 479 $[M-H]^-$, 299 [M-coniferyl alcohol]$^-$. ^{1}H-NMR (500MHz, DMSO-d_6): δ 11.90 (1H, s, OH-5), 8.17 (2H in total, d, *J*=8.5Hz, H-2', 6'), 7.09 (1H, d, *J*=2.0Hz, H-2''), 7.00 (2H in total, d, *J*=8.5, H-3', 5'), 6.94 (1H, dd, *J*=2.0, 8.1Hz, H-6''), 6.88 (1H, d, *J*=8.1Hz, H-5''), 6.40 (1H, s, H-6), 5.17(1H, d, *J*=7.7Hz, H-7''), 4.30 (1H, m, H-8''), 3.84 (3H in total, s, O-CH_3), 3.48 (1H, m, H-9''), 3,37 (1H, m, H-9''). ^{13}C-NMR (125MHz, DMSO-d_6): δ 176.4 (C-4), 159.6 (C-4'), 152.5 (C-5), 149.0 (C-7), 147.9 (C-3''), 147.4 (C-4''), 147.2 (C-2), 143.8 (C-9), 136.3 (C-3), 129.8 (C-2', 6'), 127.1 (C-1''), 124.6 (C-8), 121.9 (C-1'), 120.8 (C-6''), 115.7 (C-3', 5'), 115.6 (C-5''), 112.0 (C-2''), 104.3 (C-10), 98.2 (C-6), 77.8 (C-8''), 77.2 (C-7''), 60.2 (C-9''), 55.8 (O-CH_3).

Assay of DPPH radical scavenging activity: The antioxidative activities of compounds **1-6** and each solvent fraction was assessed on the basis of the scavenging activity of the stable DPPH free radical. Reaction mixtures containing 10 μl of test samples which were dissolved in DMSO and 190 μl of DPPH ethanolic solution (200 uM) in 96 well microtiter plates were incubated at 37°C for 30 min, and absorbances were measured at 517 nm at least in triplicate. Percent inhibition by sample treatment was determined by comparison with a DMSO treated control group. IC_{50} values denote the concentration of sample



1



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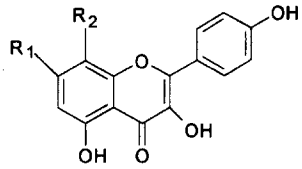
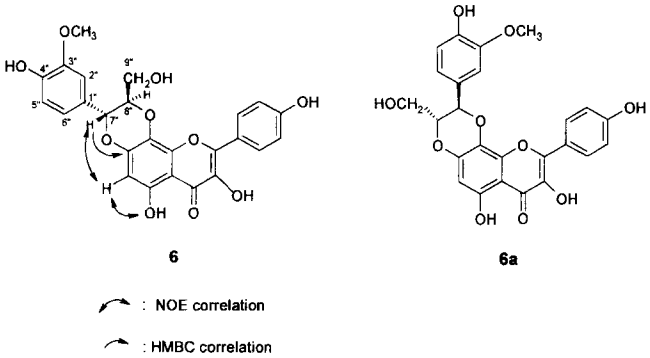


	R_1	R_2
3 :	OH	H
4 :	O-rha	H
5 :	O-rha	OH



6 6a

: NOE correlation

: HMBC correlation

required to scavenge 50% DPPH free radicals.

RESULTS AND DISCUSSION

Fractionation and separation of the acetone extract of the roots of *R. sachalinensis* led to the isolation of five phenolic compounds. The structures of these compounds were identified as gallic acid (**1**, Pang *et al.*, 1996), (-)-epi-gallocatechin 3-O-gallate (**2**, Lee *et al.*, 1992; Agrawal, 1989), kaempferol (**3**, Lee, 1995), kaempferol-7-O-α-L-rhamnopyranoside (**4**, Agrawal, 1989) and herbacetin 7-O-α-L-rhamnopyranoside (**5**, Zapesochnaya *et al.*, 1983; Agrawal, 1989).

Compound **6** showed signal pattern similar to that of compound **5** due to the presence of herbacetin moiety at δ 11.90 (1H, s, OH-5), 8.17 (2H in total, d, *J*=8.5 Hz, H-2′, 6′), 7.00 (2H in total, d, *J*=8.5 Hz, H-3′,5′), and 6.40 (1H, s, H-6) in the ^{1}H-NMR spectrum. The ^{1}H NMR spectrum of **6** also exhibited the 1,3,4-tri substituted benzene ring at 7.09 (1H, d, *J*=2.0 Hz, H-2″), 6.94 (1H, dd, *J*=2.0, 8.1Hz, H-6″), and 6.88 (1H, d, *J*=8.1Hz, H -5″), two hydroxyl bearing trans-oriented methines at δ 5.17 (1H, d, *J*=7.7 Hz, H-7″) and 4.30 (1H, m, H-8″) and a methoxyl group signal at δ 3.84 (3H in total, s, O-CH_3), a hydroxyl bearing methylene at δ 3.48 (1H, m, H-9″) and 3,37 (1H, m, H-9b″) which were proved to be α,β-substituted coniferyl alcohol moiety. The ^{13}C-NMR spectrum of **6** also exhibited addi-tional 10 carbon atoms except herbacetin moiety and their chemical shifts were iden-tical with those of coniferyl alcohol that was linked to *ortho*-related dihydroxyl group of flavonoid (Kikuchi *et al.*, 1991). These results suggested that **6** consisted of herbacetin and coniferyl alcohol via oxidative coupling. Negative FAB MS of **6** supported this fact at *m/z* 479 [M-H]$^-$ and 299 [M-coniferyl alcohol]$^-$. And the coniferyl alcohol group considered to be linked at C-7 and C-8 hydroxyl groups of herbacetin and the relative stereo-chemistry of the benzodioxane ring is proposed as depicted in **6** based on diaxial coupling of H-7″ (J=7.7 Hz). To confirm the mode of linkage, HMBC, ^{1}H-^{1}H COSY and NOESY experiments were attempted. In the ^{1}H-^{1}H COSY spectrum of **6**, the doublet (H-7″) showed correla-tion with the H-8″ multiplet at δ 4.30 (H-8″) and the signal also showed following correlation with H-9″ signals at δ 3.48. These results confirmed the assignment of each protons of coniferyl alcohol moiety unambiguously. In the Selective NOESY spectrum of **6**, irradiation at the frequency of H-6 signal (δ 6.40) showed its Selective NOE cross peak attributable to the H-7″ signal of coniferyl alcohol moiety and the 5-OH signal of herbacetin moiety. Finally, the doublet signals at δ 5.17 (1H, *J*=7.7 Hz, H-7″) showed its cross correlation with δ 149.0 (C-7) in the HMBC spectrum of **6**. Hence the structure of this compound can be shown by formula **6**. It was reported that similar compound (rhodiolin) was suggested as racemic mixture of **6** and **6a** in view of zero specific rotation, UV and ^{1}H-NMR analysis from the root of *Rhodiola rosea* (Zapesochnaya and Kurkin, 1983). But we could conclude that compound **6** named as rhodiolinin has the geometric structure of **6** on the basis of the Selective NOESY and HMBC experiment. However, it was not clear that the absolute configurations of 7″ and 8″ were αRαR (7″R8″R) or αSαS (7″S8″S).

Previously, it was reported that the BuOH fraction of the methanol extract of *R. sachalinensis* showed more potent superoxide dismutase activity and inhibitory effects

Table I. Antioxidative activities of solvent fractions from the roots of *R. sachalinensis*.

Fractions	IC_{50} (μg/ml)
MeOH ext.	20.86
Hexane fr.	66.88
CH_2Cl_2 fr.	344.33
EtOAc fr.	12.46
BuOH fr.	21.93
H_2O	22.64

Table II. Antioxidative activities of phenolic compounds from the roots of *R. sachalinensis*.

Compound	IC_{50} (μM)
Gallic acid (**1**)	28.16 ± 1.03
(-)-Epigallocatechin 3-O-gallate (**2**)	22.97 ± 1.24
Kaempferol (**3**)	50.51 ± 1.15
Kaempherol 7-O-α-L-rhamanopyranoside (**4**)	87.42 ± 2.30
Herbacetin 7-O-α-L-rhamanopyranoside (**5**)	48.34 ± 0.96
Rhodiolinin (**6**)	72.23 ± 2.32
Ascorbic acid	56.25 ± 0.92
BHA	91.15 ± 5.69
BHT	66.83 ± 7.31

Each value is the mean of at least three independent experiments ± SD. Statistical analysis was performed by using Student's T-test.

of lipid peroxidation than the non-polar solvent fraction (CH_2Cl_2 fraction) (Ryu *et al.*, 1998). In the present work, the polar solvent fraction showed strong DPPH radical scavenging activity, especially in EtOAC fraction (Table I). These results suggested that the active component of *R. sachalinensis* was hydrophilic compound like plant phenol. Hence the phenolic compounds, which were isolated from the acetone extract of the root of *R. sachalinensis*, showed strong DPPH radical scavenging activity (Table I). Especially gallic acid (**1**) and (-)-epi-gallocatechin 3-O-gallate (**2**) with IC_{50} values of 28.16 and 22.97 μM, respectively, showed potent antioxidative activities in comparison with reference antioxidants such as ascorbic acid (IC_{50} 56.25 μM), BHA (IC_{50} 91.15 μM) and BHT (IC_{50} 66.83 μM). Kaempferol (**3**), kaempferol 7-O-α-L-rhamnopyranoside (**4**) and herbacetin 7-O-α-L-rhamnopyranoside (**5**) showed moderate antioxidative activities with IC_{50} values of 50.51, 87.42, and 48.34 μM, respectively. And these phenolic compounds might be contributed to strong antioxidative activity of the roots of *R. sachalinensis*.

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