Isolation of Angiotensin Converting Enzyme (ACE) Inhibitory Flavonoids from Sedum sarmentosum

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Bioassay-guided fractionation of the EtOAc-soluble extract of Sedum sarmentosum afforded a new flavonoid, quercetin-3-O-α(6′-caffeylglucosyl)-β,1,2-rhamnoside (1), along with four known flavonoids, quercetin 3-O-α(6′-coumaroylglucosyl)-β,1,2-rhamnoside (2), isorhamnetin-3β-glucopyranoside (3), quercetin-3β-glucopyranoside (4), and kaempferol-3α-arabinopyranoside (5). Purification of these compounds was conducted with the application of various chromatographic methods. Compounds 1—5 inhibited angiotensin I converting enzyme (ACE) activity in a concentration-dependent manner. Compounds 1—5 had 50% inhibitory concentration values of 158.9 ± 11.1 μM, 351.6 ± 3.9 μM, 408.9 ± 4.6 μM, 708.8 ± 23.1 μM, and 392.8 ± 13.4 μM.

Key words Sedum sarmentosum; angiotensin converting enzyme (ACE); inhibitor

The genus Sedum (Crassulaceae) is found mainly in various East-European regions and a vast number of the species are used pharmacologically. 1) The genus Sedum is known to contain various classes of compounds such as alkaloids, 2) flavonoids, 3) and cyanogenic compounds. 4,5) Angiotensin converting enzyme (ACE) is a zinc-dipeptidyl dipeptidase that physiologically converts angiotensin I to angiotensin II, which is considered as the active constituent. 6) In addition, hepatoprotective terpenoids have been reported from Sedum sarmentosum. 7)

Angiotensin converting enzyme (ACE) is a zinc-dipeptidyl dipeptidase that physiologically converts angiotensin I to angiotensin II, which is a potent vasoconstrictor. Angiotensin II is also known to stimulate both the synthesis and release of aldosterone from the adrenal cortex, and this event increases blood pressure via sodium retention. 8) Therefore, the inhibition of ACE has been considered to be one of the effective therapeutic approaches for the treatment of cardiovascular diseases such as hypertension. As part of our search for a therapeutic approach for the treatment of high blood pressure, we have been conducting in vitro screening for the ACE inhibitory effects of various extracts from medicinal plants, and found that an ethylacetate-soluble extract of S. sarmentosum exhibited distinctive ACE inhibitory activity at the 400 μg/ml level. Bioassay-directed further purification of the extract of S. sarmentosum using various chromatographic methods afforded a new flavonoid along with four known compounds.

MATERIALS AND METHODS

Plant Material Fresh aerial parts of S. sarmentosum were collected in the Botanical Garden of Wonkwang University, Iksan, Korea, in July 2000. The plant was identified and authenticated by one of the co-authors (T.-O. Kwon). A voucher specimen (No. DH-67) was deposited in the Herbarium of the Professional Graduate School of Oriental Medicine, Wonkwang University.

Isolation of Active Principles Fresh aerial parts of S. sarmentosum (4 kg) were extracted with MeOH (61) for 3 d. The MeOH extract was concentrated, suspended in H2O, and sequentially partitioned with n-hexane, EtOAc, and BuOH. The bioactive EtOAc-soluble fraction (6.6 g) was subjected to silica gel vacuum flash column (900 ml, collecting 300 ml fractions), followed by MeOH in CH2Cl2 (900 ml, collecting 300 ml fractions), affording Fr. 1 to Fr. 15. The fraction eluted with 10% MeOH in CH2Cl2 (Fr. 10, elution volume: 2600—2900 ml, 399.3 mg) was subjected to Sephadex LH-20 column (40 g, 2.5×30 cm) chromatography with a stepwise gradient elution sequence of CH2Cl2—acetone (1:1, 300 ml), CH2Cl2—acetone (1:4, 200 ml), acetone (160 ml), acetone-MeOH (4:1, 40 ml), and MeOH (60 ml), collecting 20 ml fractions. Fractions of similar composition as determined by TLC analysis were pooled. Compounds 3 (23.5 mg) and 4 (29.3 mg) were eluted in fraction numbers 31 to 34 (elution volume: 620—680 ml), and 35 (elution volume: 680—700 ml), respectively. Further purification of the fraction numbers 26 to 30 (elution volume: 520—600 ml) using reversed-phase HPLC [C18 column (19×300 mm, 7 μm particle size), gradient from 20 to 60% acetonitrile in H2O over 30 min, 4 ml/min, detection at 254 nm] yielded compounds 3 (13.5 mg, elution volume=92.9—97.3 ml) and 5 (4.2 mg, elution volume=98.6—103.7 ml).

The fraction eluted with 20% MeOH in CH3Cl (Fr. 12, elution volume: 3200—3500 ml, 397.2 mg) was subjected to Sephadex LH-20 column (40 g, 2.5×30 cm) chromatography with a stepwise gradient elution sequence of H2O (140 ml), MeOH-H2O (1:9, 160 ml), MeOH-H2O (1:4, 60 ml), MeOH-H2O (1:1, 140 ml), and MeOH (60 ml), collecting 20 ml fractions. Fractions of similar composition as determined by TLC analysis were pooled. Further purification of the fraction numbers 21 to 23 (elution volume: 420—460 ml, 70 mg) using reversed-phase HPLC [BDS C18 column (10×250 mm; 8 μm particle size), gradient from 15 to 30% acetonitrile in H2O over 60 min, 2 ml/min, detection at 254 nm] yielded compounds 1 (16.8 mg, elution volume...
The optical rotation was recorded on an Optical Activity AA-10 Automatic Polarimeter. FAB-MS data were obtained on a JEOL JMS HX-110 spectrometer using 3-nitrobenzyl alcohol as a matrix. ESI-MS data were obtained on a Macro Mass Quatro LC with electrospray ionization. NMR spectra (1D and 2D) were recorded in acetone-d$_6$ or MeOH-d$_4$ using a JEOL Eclipse-500 MHz spectrometer (500 MHz for $^1$H and 150 MHz for $^{13}$C), and chemical shifts were referenced relative to the corresponding residual solvent signals. HMOC and HMBC data were optimized for $^1$J$_{CH}$=140 Hz and $^3$J$_{CH}$=8 Hz, respectively.

**Measurement of ACE Activity** Plasma ACE activity was determined in rat plasma by a method previously described. Briefly, plasma (10 μl) was incubated with 490 or 480 μl of assay buffer containing 5 mmol/l Hip–His–Leu in 40 mmol/l sodium borate buffer and 0.9 mol/l NaCl, and 10 μl of sample, pH 8.3, for 15 min at 37°C. The reaction was stopped by the addition of 1.2 ml of 3.4 N NaOH. The product, His–Leu, was measured fluorometrically at 365 nm excitation and 495 nm emission with a fluorescence spectrophotometer (Hitachi, model F-2000, Tokyo, Japan) as follows. After 100 μl of O-phthalaldehyde (20 mg/ml) in methanol was added to the reaction solution for 10 min, the solution was acidified with 200 μl of 3 N HCl and centrifuged at 3000 rpm for 10 min at room temperature. To correct the intrinsic fluorescence of the plasma, a zero time blank was prepared by adding plasma after NaOH treatment. The 50% inhibitory concentrations (IC$_{50}$) were determined for compounds 1–5 and a positive control, captopril, using linear regression analysis. Three separate determinations were conducted for each compound.

**RESULTS AND DISCUSSION**

The molecular formula of 1 was determined as C$_{36}$H$_{36}$O$_{19}$ by high resolution FAB-MS, which showed a quasimolecular ion peak at m/z 773.1929 [M+H]$^+$. The $^1$H- and $^{13}$C-NMR data of 1 were almost identical with those of known compound 2, except for the presence of $^1$H- and $^{13}$C-signals corresponding to a 1,3,4-trisubstituted aromatic ring instead of the 1,4-disubstituted aromatic ring found in 2. A combination of $^1$H- $^{13}$C COSY and HMOC experiments allowed unambiguous assignments of all proton and carbon signals as well as the connectivities among quercetin, caffeoyl, and two sugar moieties (Table 1). Therefore, compound 1 was elucidated as quercetin-3-O-α-(6"-caffeoylglucosyl-β-1,2-rhamnoside). LR-ESI-MS $m/z$: 771 [M–H]; LR-FAB-MS $m/z$: 773 [M+H]$^+$; HR-FAB-MS $m/z$: 773.1929 (Caled. for C$_{36}$H$_{36}$O$_{19}$: 773.1929). NMR data are listed in Table 1. Compounds 2–5 were determined to be quercetin-3-O-α-(6"-p-coumaroylglucosyl-β-1,2-rhamnoside) (2), isorhamnetin-3-β-glucopyranoside (3), quercetin-3-β-glucopyranoside (4), and kaempferol-3-α-arabinopyranoside (5), respectively, by analysis of various spectral data (MS, $^1$H-, $^{13}$C-NMR), and by comparing their spectral data with the literature values (Fig. 2). Copies of the original spectra are obtainable from the author of correspondence.

### Table 1. NMR Spectral Data for Compound 1 (CD$_3$OD)

<table>
<thead>
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<th>No.</th>
<th>$^{13}$C-NMR δ [(ppm), mult.]</th>
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<tr>
<td>1</td>
<td>158.4 (s)</td>
<td>158.4 (s)</td>
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<td>2</td>
<td>136.8 (s)</td>
<td>179.7 (s)</td>
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<td>106.0 (s)</td>
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<td>6</td>
<td>123.0 (s)</td>
<td>117.0 (d)</td>
<td>2, 3’, 4’, 6’</td>
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<tr>
<td>7</td>
<td>146.3 (s)</td>
<td>149.7 (s)</td>
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**Fig. 1. Angiotensin Converting Enzyme Inhibitory Effect by Hexane (HX), Ethylacetate (EA), Butanol (BU), and Water (H$_2$O)-Extracts of Schedum sarmentosum**

VEH denotes vehicle. n=4, each experiment.

As part of our search for a therapeutic approach for the treatment of high blood pressure, solvent-extracts from *S. sarmentosum* were screened for their inhibitory effects on ACE. Among the tested extracts, the EtOAc-soluble extract of *S. sarmentosum* was found to exhibit distinctive ACE inhibitory activity at the 400 μg/ml level (Fig. 1). Compounds 1–5 isolated from *S. sarmentosum* as active principles inhibited the ACE activity in a dose-dependent manner, and the 50% inhibitory concentration (IC$_{50}$) values for compounds 1–5 were determined to be 158.9±11.1 μM, 351.6±3.9 μM,
group of Arg, a pocket that accommodates a hydrophobic side chain of C-terminal amino acid residues, and a zinc ion. The zinc ion coordinates to the carbonyl of the penultimate peptide bond of the substrate, whereby the carbonyl group becomes polarized and is subjected to a nucleophilic attack. Therefore, some flavonoids were suggested to show in vitro activity via the generation of chelate complexes within the active center of ACE.\(^{16}\) Free hydroxyl groups of phenolic compounds have also been suggested to be important structural moieties to chelate the zinc ions, thus inactivating the ACE activity.\(^{18}\) Since compounds 1—5 contain aromatic hydroxyl groups, these hydroxyl groups may exhibit ACE inhibitory activity due to the generation of chelate complexes with zinc ions within the active center of ACE. Thus, compounds 1—5 seem to have similar or stronger levels of inhibitory activities toward ACE as compared to previously reported ACE-inhibitory flavonoids.

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**REFERENCES**


<table>
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<th>Compounds</th>
<th>IC(_50) ((\mu g))\textsuperscript{a})</th>
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<tr>
<td>1</td>
<td>158.9±11.1</td>
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<tr>
<td>2</td>
<td>351.6±3.9</td>
</tr>
<tr>
<td>3</td>
<td>408.9±4.6</td>
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<tr>
<td>4</td>
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\(a\) IC\(_{50}\) values are mean±S.E.M. (n=3).