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‴-caffeoylglucosyl-β-1,2-rhamnoside) (1), along with four known flavonoids, 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). 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 pharmaceutically.[1)] The genus *Sedum* is known to contain various classes of compounds such as alkaloids,[2)] flavonoids,[3)] and cyanogenic compounds.[4,5)] *Sedum sarmentosum* Bunge, in particular, has been used for the treatment of chronic viral hepatitis in Asian countries, and sarmentosin 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 (6 l) for 3 d. The MeOH extract was concentrated, suspended in H_2O, 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 (100 g, 4.5×10 cm, 15—40 μm particle size) chromatography. The column was eluted with a stepwise gradient with 20% hexane in CH_2Cl_2 (900 ml, collecting 300 ml fractions), followed by MeOH in CH_2Cl_2 (0% 600 ml, 5% 600 ml, 7% 300 ml, 10% 600 ml, 20% 600 ml, 30% 900 ml, and 50% 600 ml, collecting 300 ml fractions), affording Fr. 1 to Fr. 15. The fraction eluted with 10% MeOH in CH_2Cl_2 (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 CH_2Cl_2–acetone (1 : 1, 300 ml), CH_2Cl_2–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 [C_{18} column (19×300 mm, 7 μm particle size), gradient from 20 to 60% acetonitrile in H_2O over 30 min, 4 ml/min, detection at 254 nm] yielded compounds **3** (13.5 mg, elution volume=92.2—97.3 ml) and **5** (4.2 mg, elution volume=98.6—103.7 ml).

The fraction eluted with 20% MeOH in CH_2Cl_2 (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 H_2O (140 ml), MeOH–H_2O (1 : 9, 160 ml), MeOH–H_2O (1 : 4, 60 ml), MeOH–H_2O (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 C_{18} column (10×250 mm; 8 μm particle size), gradient from 15 to 30% acetonitrile in H_2O over 60 min, 2 ml/min, detection at 254 nm] yielded compounds **1** (16.8 mg, elution volume

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=41.2—44.0 ml) and **2** (6.0 mg, elution volume=49.2—49.8 ml).

Determination of Chemical Structure 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. HMQC and HMBC data were optimized for $^1J_{CH}$=140 Hz and $^nJ_{CH}$=8 Hz, respectively.

Measurement of ACE Activity Plasma ACE activity was determined in rat plasma by a method previously described.[9,10] 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 fluorimetrically 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 time zero 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–^{1}H COSY and HMQC 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 (Calcd. for $C_{36}H_{37}O_{19}$, 773.1929); $[\alpha]_D^{25}=-147.6°$ (*c*=0.21, MeOH); 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**),[11] isorhamnetin-3-β-glucopyranoside (**3**),[12] quercetin-3-β-glucopyranoside (**4**),[13] and kaempferol-3-α-arbinopyranoside (**5**),[12] 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).[11—13] Copies of the original spectra are obtainable from the author of correspondence.

Table 1. NMR Spectral Data for Compound **1** (CD_3OD)

No.	^{13}C-NMR δ [(ppm), mult.]	^{1}H-NMR δ [(ppm), mult., *J* (Hz)]	HMBC (H#→C#)
2	158.4 (s)		
3	136.8 (s)		
4	179.7 (s)		
5	163.1 (s)		
6	99.8 (d)	6.15 (d, 1.9)	5, 7, 8, 10
7	165.8 (s)		
8	94.8 (d)	6.20 (d, 1.9)	6, 7, 9, 10
9	158.5 (s)		
10	106.0 (s)		
1′	123.0 (s)		
2′	117.0 (d)	7.34 (d, 2.3)	2, 3′, 4′, 6′
3′	146.3 (s)		
4′	149.7 (s)		
5′	116.4 (d)	6.90 (d, 8.3)	1′, 3′, 4′
6′	122.86 (d)	7.24 (dd, 8.3, 2.3)	2, 2′, 4′
1″	102.7 (d)	5.69 (br s)	3, 2″, 3″
2″	83.6 (d)	4.34 (d, 2.3)	3″, 4″, 1‴
3″	71.8 (d)	3.87 (dd, 10.0, 3.7)	4″
4″	73.6 (d)	3.35 (dd, 9.8, 9.8)[a]	6″
5″	71.9 (d)	3.69 (m)	3″, 4″
6″	17.7 (q)	1.05 (d, 6.0)	4″, 5″
1‴	107.2 (d)	4.42 (d, 7.8)	2″, 3‴
2‴	75.2 (d)	3.27 (dd, 9.2, 9.2)[a]	1‴, 3‴
3‴	77.7 (d)	3.39 (dd, 9.2, 9.2)[a]	2‴, 4‴
4‴	71.7 (d)	3.30 (dd, 9.3, 9.3)[a]	2‴, 6‴
5‴	75.4 (d)	3.41 (m)	4‴, 5‴
6‴	64.2 (t)	4.14 (dd, 6.0, 11.9) 4.44 (dd, 2.3, 11.9)	4‴, 5‴, 1⁗
1⁗	168.9 (s)		
2⁗	114.7 (d)	6.01 (d, 15.6)	1⁗, 4⁗
3⁗	147.0 (d)	7.37 (d, 15.6)	1⁗, 2⁗, 4⁗, 9⁗
4⁗	127.6 (s)		
5⁗	115.0 (d)	6.85 (d, 2.3)	6⁗, 7⁗, 9⁗
6⁗	146.6 (s)		
7⁗	149.4 (s)		
8⁗	116.3 (d)	6.65 (d, 7.8)	4⁗, 6⁗
9⁗	122.94 (d)	6.74 (dd, 7.8, 2.3)	3⁗, 5⁗, 7⁗

a) *J* values were determined by *J*-resolved spectrum.

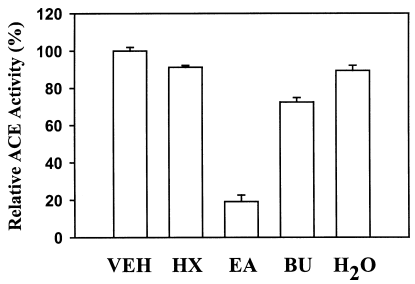

Fig. 1. Angiotensin Converting Enzyme Inhibitory Effect by Hexane (HX), Ethylacetate (EA), Butanol (BU), and Water (H_2O)-Extracts of *Sedum sarmentosum*

VEH denotes vehicle. *n*=4, each experiment.

As part of our search for a therapeutic approach for the treatment of high blood pressure, solvent-exracts 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,

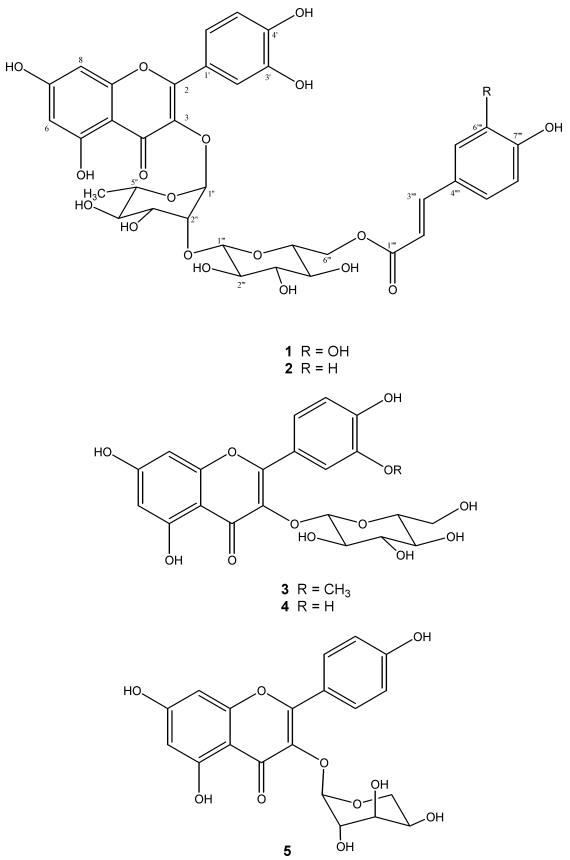

Fig. 2. Chemical Structures of Angiotensin Converting Enzyme Inhibitory Components (**1**—**5**) Isolated from *Sedum sarmentosum*

Table 2. Angiotensin Converting Enzyme Inhibitory Activities of Compounds **1**—**5**

Compounds	IC_{50} (μM)[a]
1	158.9±11.1
2	351.6±3.9
3	408.9±4.6
4	708.8±23.1
5	392.8±13.4
Captopril	0.02

a) IC_{50} values are mean±S.E.M. (n=3).

408.9±4.6 μM, 708.8±23.1 μM, and 392.8±13.4 μM, respectively (Table 2). Among the isolated compounds from *S. sarmentosum*, **1** was two-fold more active than its closely related compound **2**. This observation suggested that the presence of a phenolic hydroxyl group at the C-6″″ position greatly enhanced the inhibitory activity of **1**. Several flavonoids, including compound **4**, have been reported to inhibit ACE activity by 60—90% at a concentration of 0.33 mg/ml.[8,14,15]

ACE is a zinc-containing peptidyl dipeptide hydrolase.[16] The active site of ACE is known to consist of three parts; a carboxylate binding functionality such as the guanidinium 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.[17] 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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