Sesquiterpene lactones and scopoletins from *Artemisia scoparia* Waldst. & Kit. and their angiotensin I-converting enzyme inhibitory activities

Jeong-Yong Cho, Seung-Jae Jeong, Hee La Lee, Kyung-Hee Park, Do Young Hwang, Sun-Young Park, Yu Geon Lee¹, Jae-Hak Moon¹, and Kyung-Sik Ham*

Department of Food Biotechnology and Solar Salt Research Center, Mokpo National University, Muan, Jeonnam 58554, Korea

¹Department of Food Science & Technology, and Functional Food Research Center, Chonnam National University, Gwangju 61186, Korea

Received April 29, 2016
Revised July 15, 2016
Accepted August 12, 2016
Published online December 31, 2016

*Corresponding Author
Tel: +82-61-450-2425
Fax: +82-61-454-1521
E-mail: ksham@mokpo.ac.kr

pISSN 1226-7708
eISSN 2092-6456

© KoSFoST and Springer 2016

Abstract Ten compounds, including a new guaiane-type sesquiterpene lactone, were isolated from the aerial parts of *Artemisia scoparia*. The structure of the new compound was determined to be 5-hydroxyguaia-3(4),11(13),10(14)-trien-6 α ,12-olide, named scoparanolide. Six known sesquiterpene lactones [estafiatone, 3 β ,4 α -dihydroxyguaia-11(13),10(14)-dien-6 α ,12-olide, estafiatin, preeupatundin, 3 β -hydroxycostunolide, and ludovicin B] and three known coumarin derivatives (scopoletin, scoparone, and isofraxidin) were identified by nuclear magnetic resonance and electrospray ionization mass spectroscopy. Six known sesquiterpene lactones were found for the first time in this plant. The angiotensin I-converting enzyme inhibitory activities of coumarin derivatives and scopoletins were significantly higher compared to those of sesquiterpene lactones and quercetin.

Keywords: angiotensin I-converting enzyme inhibitor, *Artemisia scoparia*, sesquiterpene lactone, scoparanolide, scopoletin

Introduction

Artemisia scoparia (redstem wormwood), the family Asteraceae, is widely distributed in Asian countries, including China, Korea, and Japan (1,2). *A. scoparia* is abundantly found in the coastal salt marshes in Korea (3). This plant has a unique aroma and bitter taste and is used as a vegetable and medicinal herb in Korea. Several studies have reported that *A. scoparia* has various health-promoting effects, including antioxidant, anti-inflammatory, antiatherogenic, anticancer, liver protection, antinociceptive, and antipyretic activities (4–11). In addition, *A. scoparia* reduces non-alcoholic fatty liver disease by enhancing hepatic insulin and AMP-activated protein kinase (AMPK) signaling in diet-induced obese mice (12). Diverse chemical constituents, such as flavonoids, scopariachromane, coumarins, sterols, and essential oils, have been found in *A. scoparia* (1,2,5,7,9,13). We previously reported that *A. scoparia* reduces blood pressure in spontaneously hypertensive rats via the inhibition of plasma angiotensin I-converting enzyme (ACE) activity, enhancement of vascular endothelial growth factor expression, and the suppression of ras homolog gene family member A expression, resulting in the reduction of oxidative stress and inflammation (14). In addition, seven ACE inhibitors, including 4-*O*- β -D-glucopyranoyl (*E*)-4-hydroxy-

3-methylbut-2-enyl benzoate, eugenol 2 *O*- β -D-glucopyranoside, and 4-(*O*- β -D-glucopyranoyl)-3-(3-methyl-2-butenyl)acetophenone, have been found in the aerial parts of *A. scoparia* (14). In the course of investigations on ACE inhibitors, 10 compounds, including seven sesquiterpene lactones and three scopoletin derivatives, were additionally isolated. In this study, we report the isolation and structural determination of seven sesquiterpene lactones and three scopoletin derivatives from the hot water extracts of the aerial parts of *A. scoparia*. In addition, the ACE inhibitory activities of the isolated compounds from *A. scoparia* aerial parts were evaluated.

Materials and Methods

Chemicals and materials The aerial parts of *A. scoparia* were collected in May 2013 in Shinan County, Korea. After washing, the aerial parts were steamed for 2 min, dried at 45°C for 2 days, and the dried samples were stored at –20°C until used. Rabbit lung acetone powder, *N*-hippuryl-L-histidyl-L-leucine (HHL), hippuric acid, and quercetin were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All solvents used for analyses were of high-performance liquid chromatography (HPLC) grade.

Extraction, solvent fractionation, and isolation The dried aerial parts (1.6 kg) of *A. scoparia* were extracted with distilled water (40 L) for 30 min using autoclave (HS-196; Hansin Medical Co., Ltd., Incheon, Korea) and filtered through No. 2 filter paper (Whatman, Maidstone, UK). The residues were repeatedly extracted with distilled water (20 L) and filtered. The hot water-extracted solutions were freeze-dried (LP10; Ilshin Lab Co., Ltd., Seoul, Korea) to obtain the hot water extract powder (461 g) of *A. scoparia*. The H₂O suspension (3 L) of the hot water extracts was successively partitioned with *n*-hexane (32 L, three times), chloroform (CHCl₃; 32 L, three times), ethyl acetate (EtOAc; 32 L, three times), and water-saturated *n*-butanol (BuOH; 32 L, three times). The fractions were concentrated at 38°C under vacuum to afford *n*-hexane (0.8 g), CHCl₃ (2.1 g), EtOAc (34.3 g), BuOH (86.2 g), and H₂O fractions. The CHCl₃ and EtOAc fractions (36.4 g) were loaded on a silica gel (Kiesel gel 60, 70–230 mesh; Merck, Darmstadt, Germany) column (800 g, 6.0 cm×30 cm) and eluted with CHCl₃/MeOH (9:1→7:3→5:5→3:7→0:10, v/v, each 8 L) to afford 11 active fractions (A–K). Fraction A (CHCl₃/MeOH=9:1, v/v; 2.74 g) was re-fractionated using a silica gel column (54.8 g, 2.5 cm×37 cm) by eluting with a series of solvent mixtures of *n*-hexane/EtOAc (8:2→6:4→4:6→2:8→0:10, v/v; each 250 mL) to afford six subfractions A1–A6. Subfraction A3 (*n*-hexane/EtOAc=8:2, v/v; 743.5 mg) was purified by HPLC with a Spherisorb S5 ODS2 column (10 mm×250 mm, 5 µm; Waters, Milford, MA, USA; mobile phase: 45% acetonitrile (MeCN), flow rate: 3.0 mL/min, and wavelength: 220 nm) to yield **1** (*t_R* 14.2 min, 18.0 mg), **2** (*t_R* 15.5 min, 2.0 mg), **3** (*t_R* 16.3 min, 1.8 mg), **4** (*t_R* 17.7 min, 18.0 mg), **5** (*t_R* 24.4 min, 1.5 mg), and **6** (*t_R* 26.5 min, 5.8 mg). Fraction A5 (*n*-hexane/EtOAc=6:4, v/v; 456.7 mg) was fractionated in an ODS (70–230 mesh; YMC, Tokyo, Japan) column (35 g, 3.0 cm×16 cm) chromatography by eluting with H₂O/MeOH (8:2, 6:4, 4:6, and 0:10; v/v; each 250 mL) to yield **7** (H₂O/MeOH=6:4, v/v; 20.5 mg). Subfraction A6 (*n*-hexane/EtOAc=2:8, v/v; 399.0 mg) was subjected to a chromatography using a Sephadex LH-20 (25–100 mesh; GE Healthcare Bio-Sciences AB, Uppsala, Sweden) column (2.0 cm×145 cm, total volume: 250 mL) and eluted with 100% MeOH to yield **8** [elution volume/total volume (Ve/Vt)=1.16–1.20, 63.1 mg] and **9** (Ve/Vt=1.21–1.24, 32.9 mg).

Fraction C (CHCl₃/MeOH=9:1, v/v; 2.1 g) was loaded onto a silica gel column (60 g, 2.5 cm×25 cm) and further fractionated by eluting with CHCl₃/MeOH=95:5 and 90:10, v/v; each 600 mL) to afford 11 subfractions C1–C11. Subfraction C6 (CHCl₃/MeOH=90:10, v/v; 61.1 mg) was subjected to a semi-preparative ODS HPLC (column, Spherisorb S5 ODS2 10 mm×250 mm, 5 µm, Waters; mobile phase: 20% MeCN, flow rate: 3.0 mL/min, and wavelength: 245 nm) to yield **10** (*t_R* 18.4 min, 18.0 mg).

The fractions obtained after column chromatography were analyzed by thin-layer chromatography (TLC). The fractions were loaded on silica gel TLC plates (silica gel 60 F254, thickness: 0.25 mm; Merck) and developed with a mixture of CHCl₃/MeOH=7:3 (v/v). The spots were detected by UV illumination at 254 nm and spraying 1% cerium IV sulfate ethanol solution.

Structural analysis Nuclear magnetic resonance (NMR) spectra were recorded using ^{unity}INNOVA 500 and 600 spectrometers (Varian, Walnut Creek, CA, USA). The isolated compounds were dissolved with deuterated chloroform (CDCl₃) or methanol-*d*₄ (CD₃OD) containing tetramethylsilane (TMS). The mass spectra were acquired on a hybrid ion trap/time-of-flight mass spectrometer (SYNAPT G2; Waters) coupled with an electrospray ionization (ESI) source (ESI-MS).

Compound 1: [α]_D²⁵ +0.93 (c 0.05, CHCl₃); ¹H- and ¹³C-NMR data shown in Table 1 and 2; ESI-MS (positive) *m/z* 247.10 [M+H]⁺.

Compound 2: [α]_D²⁵ 0.45 (c 0.03, CHCl₃); ¹H- and ¹³C-NMR data shown in Table 1 and 2; ESI-MS (positive) *m/z* 265.0 [M+H]⁺.

Compound 3: [α]_D²⁵ +1.19 (c 0.02, CHCl₃); ¹H- and ¹³C-NMR data shown in Table 1 and 2; HR-ESI-MS (positive) *m/z* 269.1147 [M+Na]⁺ (calculated for C₁₅H₁₈O₃Na, *m/z* 269.1154, and 0.7 mDa)

Compound 4: [α]_D²⁵ +0.2 (c 0.10, CHCl₃); ¹H- and ¹³C-NMR data shown in Table 1 and 2; ESI-MS (positive) *m/z* 269.0 [M+Na]⁺.

Compound 5: [α]_D²⁵ +12.8 (c 0.017, CHCl₃); ¹H- and ¹³C-NMR data shown in Table 1 and 2; ESI-MS (positive) *m/z* 269.0 [M+Na]⁺.

Compound 6: [α]_D²⁵ +58.5 (c 0.014, CHCl₃); ¹H- and ¹³C-NMR data shown in Table 1 and 2; HR-ESI-MS (positive) *m/z* 269.1259 [M+Na]⁺ (calculated for C₁₅H₁₈O₃Na, *m/z* 269.1254, and 0.5 mDa).

Compound 7: ¹H-NMR (400 MHz, CD₃OD) δ 3.80 (3H, s, –OCH₃), 6.19 (H, d, *J*=9.6 Hz, H-3), 6.76 (1H, s, H-8), 7.20 (1H, s, H-5), 7.89 (1H, d, *J*=9.6 Hz, H-4); ¹³C-NMR (100 MHz, CD₃OD) δ 56.4 (–OCH₃), 166.3 (C-2), 113.4 (C-3), 145.9 (C-4), 107.5 (C-5), 143.3 (C-6), 149.2 (C-7), 103.5 (C-8), 149.2 (C-9), 113.4 (C-10); ESI-MS (positive) *m/z* 215.1 [M+Na]⁺.

Compound 8: ¹H-NMR (400 MHz, CD₃OD) δ 3.87 (3H, s, 7-OCH₃), 3.92 (3H, s, 6-OCH₃), 6.26 (H, d, *J*=9.6 Hz, H-3), 7.88 (1H, d, *J*=9.6 Hz, H-4), 7.13 (1H, s, H-5), 6.98 (1H, s, H-8); ¹³C-NMR (100 MHz, CD₃OD) δ 56.9 (2×–OCH₃), 163.8 (C-2), 110.0 (C-3), 145.9 (C-4), 113.6 (C-5), 148.2 (C-6), 154.8 (C-7), 101.0 (C-8), 151.3 (C-9), 113.1 (C-10); ESI-MS (positive) *m/z* 229.1 [M+Na]⁺.

Compound 9: ¹H-NMR (400 MHz, CD₃OD) 3.87 (3H, s, 7-OCH₃), 3.92 (3H, s, 6-OCH₃), 6.26 (1H, d, *J*=9.4 Hz, H-3), 7.13 (1H, s, H-5), 7.88 (1H, d, *J*=9.4 Hz, H-4); ¹³C-NMR (100 MHz, CD₃OD) δ 56.9 (2×–OCH₃), 101.0 (C-8), 110.0 (C-3), 113.1 (C-10), 113.6 (C-5), 145.9 (C-4), 148.2 (C-6), 151.3 (C-9), 154.8 (C-7), 163.8 (C-2); ESI-MS (positive) *m/z* 245.1 [M+Na]⁺.

Compound 10: ¹H- and ¹³C-NMR data shown in Table 1 and 2; HR-ESI-MS (positive) *m/z* 287.1259 [M+Na]⁺ (calculated for C₁₅H₂₀O₄Na, *m/z* 287.1259, and 0 mDa).

Determination of ACE inhibitory activity According to Cushman and Cheung (15), the ACE inhibitory activities were assayed by measuring the hippuric acid released from HHL with slight modification. The samples (20 µL) containing the fractions and the isolated compounds were mixed with 130 µL of 0.1 M sodium borate buffer (pH 8.3) containing 300-mM NaCl and 50 µL of 5-mM HHL, which was pre-incubated at 37°C for 10 min. The mixture was added to ACE extract (100 µL) of rabbit lung acetone powder. After incubation at

37°C for 90 min, the reaction was stopped by adding 2 N HCl (0.2 mL) and partitioned with 2 mL of EtOAc. The EtOAc layer (1.5 mL) was concentrated *in vacuo* and the concentrate was dissolved in 1 M NaCl (1 mL). The absorbance (A) was measured at a wavelength of 228 nm using a spectrophotometer (HP8542A; GMI, Inc., Ramsey, MN, USA). Quercetin was used as a positive control. The absorbance (B) of quercetin, fractions, and isolated compounds were measured without the ACE extract, as described in the above procedure. The ACE inhibitory activities of the samples were determined as a percentage decreased from absorbance of a control test. The ACE inhibitory activities of the isolated compounds at a concentration of 1 mM were analyzed. ACE inhibitory activity (%) = [(control - (A - B)) / control] × 100.

Statistical analysis Data are expressed as means with standard deviations (SD) using the SPSS (IBM, Armonk, NY, USA) 19.0 software package. The significance of difference was assessed by one-way analysis of variance, followed by Tukey's multiple comparison test. A value of $p < 0.05$ was considered to indicate significance.

Results and Discussion

Isolation and structural determination The dried aerial parts of *A. scoparia* were extracted with hot water, and the H₂O suspension of the hot water extracts were partitioned to obtain *n*-hexane, CHCl₃, EtOAc, BuOH, and H₂O fractions. The hot water extract and fractions showed ACE inhibitory activities (Fig. 1). In particular, the CHCl₃ fraction (30.6%) exhibited the highest ACE inhibitory activity. The ACE inhibitory activity (23.7%) of EtOAc fraction was similar to that of the hot water extracts (26.1%). BuOH and H₂O fractions showed very low ACE inhibitory activities (<2%) (Fig. 1). Therefore, we attempted to isolate and identify the ACE inhibitors from the CHCl₃ and EtOAc fractions of *A. scoparia* aerial parts. The CHCl₃ and EtOAc fractions were combined and fractionated by silica gel column chromatography. Eleven fractions were grouped by TLC profile and evaluated by ACE inhibitory assay. Fractions C and D showed the highest ACE inhibitory activities. In addition, fraction A had a slightly higher ACE inhibitory activity than the other fractions. We previously reported the isolation and identification of seven ACE inhibitors, including a new compound [4-*O*-β-D-glucopyranoyl (*E*)-4-hydroxy-3-methylbut-2-enyl benzoate] from fractions C and D (14). In this study, we additionally isolated 10 compounds (**1**–**10**) from fractions A and C by column chromatography and HPLC (Fig. 2). Among them, three known compounds were identified as scopoletin (**7**) (16), scoparone (**8**) (17), and isofraxidin (**9**) (18) (Fig. 3) via a comparison of NMR and MS spectroscopic data previously reported.

Molecular weight of **1** was 246, as established by the pseudomolecular ion peak at m/z 247.10 [M+H]⁺ in the ESI-MS (positive) spectrum. The ¹H-NMR (500 MHz, CD₃OD) spectrum of **1** exhibited proton signals of two *sp*² methylenes at δ 6.30 (1H, d, J =3.5

Fig. 1. The angiotensin I-converting enzyme (ACE) inhibitory activity of the hot water extract of *Artemisia scoparia* aerial parts and fractions obtained after its solvent fractionation. Reaction solution containing 0.1 M sodium borate buffer (pH 8.3) that contains 0.3 M NaCl and 5 mM *N*-hippuryl-L-histidyl-L-leucine (HHL) solution as a substrate, and rabbit lung acetone powder solution as an ACE source was incubated at 37°C for 1 h. The absorbance of released hippuric acid was determined at 228 nm. The hot water extract and fractions (200 μg dry wt. eq.) were assayed. Values are expressed as means ± SD ($n=3$). Different letters indicate a significant difference among compounds at $p < 0.05$.

Hz, H-13a), 5.58 (1H, d, J =3.5 Hz, H-13b), 5.02 (1H, br. s, H-14a), 4.69 (1H, br. s, H-14b) and five methine proton signals at δ 3.10 (1H, td, J =8.5, and 2.0 Hz, H-1), 2.30 (1H, d, J =7.5 Hz, H-4), 2.31 (1H, m, H-5), 4.40 (1H, dd, J =8.8 and 8.8 Hz, H-6), and 3.00 (1H, dddd, J =8.0, 8.0, 3.0, and 3.0 Hz, H-7) (Table 1). In addition, three methylene proton signals at δ 2.16 (2H, t, J =8.5 Hz, H-2), 2.35 (1H, m, H-8a), 1.48 (1H, m, H-8b), 2.63 (1H, m, H-9a), and 2.22 (1H, m, H-9b) and one methyl proton signal at δ 1.28 (3H, d, J =6.5 Hz, H-15) were observed. The ¹H-NMR spectrum was in agreement with the ¹³C-NMR (125 MHz, CD₃OD) spectrum, which contained a total of 15 carbon signals, including one ketone carbon at δ 219.2 (C-3), one ester carbon at δ 169.9 (C-12), and two quaternary *sp*² carbons at δ 148.7 (C-10) and 138.7 (C-11) (Table 2). Therefore, compound **1** was suggested to be a sesquiterpene lactone containing a ketone and two unsaturated methylene groups. The connectivity of **1** was further confirmed by the heteronuclear single quantum correlation (HSQC), proton–proton correlation spectroscopy (¹H–¹H COSY), and heteronuclear multiple-bond correlation (HMBC) experiments. Correlations of H-1/H-2, H-1/H-5, H-5/H-4, H-5/H-6, H-6/H-7, H-7/H-8, and H-8/H-9 were observed in the ¹H–¹H COSY spectrum (Fig. 3, bold lines), indicating that the skeleton structure of **1** was guaian-6,12-olide. The long-range proton–carbon correlations were observed in the HMBC spectrum (indicated by arrows in Fig. 3). In particular, the correlations of H-1, H-5, and H-15 to C-3 in the HMBC spectrum (indicated by arrows in Fig. 3) indicated that a ketone group was located at C-3 of guaian-6,12-olide. In addition, the correlations of H-13a to C-1 and C-9 and H-14a to C-7 and C-12 in the HMBC experiment (Fig. 2)

Fig. 2. Purification procedure for isolated compounds from the aerial parts of *Artemisia scoparia*. CC: column chromatography.

established that two unsaturated methylene groups were of the C-10 and C-11 of guaia-6,12-olide, respectively. In the nuclear overhauser effect (NOE) experiments of **1**, the irradiation of H-1 at δ 3.10 enhanced the signals of H-5 at δ 2.31, H-7 at δ 3.00, and H-15 at δ 1.28 suggesting that H-1, H-5, H-7, and H-15 were in a same configuration. The absolute stereostructure was determined (Fig. 3) by a comparison of specific rotation of that reported in literature (19). Therefore, the structure of compound **1** was identified as 3-oxo-10(14),11(13)-guaidiene-6 α ,12-olide (estafiatone) (Fig. 3).

The ^1H - and ^{13}C -NMR spectra of **2–6** were similar to those of **1** (Table 1 and 2). The α,β -unsaturated- γ -lactone ring moiety was identical, and the hydroxyl groups, double bonds, and their positions slightly differed, which were suggested to be sesquiterpene lactones. The structures of **2–6** were determined using 2D (HSQC, HMBC, ^1H - ^1H COSY, and NOESY)-NMR and MS spectroscopic data. Consequently, these compounds, with the exception of **3**, were identified as 3 β ,4 α -dihydroxyguaia-11(13),10(14)-dien-6 α ,12-olide (**2**) (20), estafiatin (**4**) (21), preeupatundin (**5**) (22), and 3 β -hydroxycostunolide (**6**) (23) (Fig. 3) by a comparison of NMR and MS spectroscopic data previously reported.

The molecular formula of **3** was $\text{C}_{15}\text{H}_{18}\text{O}_3$, as established by the pseudomolecular ion peak at m/z 269.1147 [$\text{M}+\text{Na}$] $^+$ (calculated for $\text{C}_{15}\text{H}_{18}\text{O}_3\text{Na}$, m/z 269.1154, and 0.7 mDa) in the ESI-MS (positive)

spectrum. The ^1H - and ^{13}C -NMR spectra of **3** showed one olefinic double bond proton [δ 5.70 (1H, m, H-3)] and two carbon signals [δ 129.5 (C-3) and 141.2 (C-4)] and one oxygenated quaternary sp^3 carbon signal [δ 85.2 (C-5)] instead of ketone- and methine-related proton and carbon signals in those of **1** (Table 1 and 2). Therefore, the structure of **3** was proposed to be guaiaatrienolide coupled with a hydroxyl group. In addition, the proposed structure of **3** was determined to be 5-hydroxyguaia-3(4),11(13),10(14)-trien-12,6 α -olide by HSQC, ^1H - ^1H COSY (indicated by bold lines in Fig. 3), and HMBC (indicated by arrows in Fig. 3). The planar structure of **3** was unambiguously identified as 5-hydroxyguaia-3(4),11(13),10(14)-trien-12,6-olide, which is a new compound named scoporanolide (Fig. 3). Correlations of H-1 and H-6 observed in the NOESY spectrum and the coupling constant value (8.4 Hz) of H-6 indicated that H-1 and H-6 had the same configuration and H-7 configuration was different from those of H-1 and H-6. However, the structural determination of the absolute stereochemistry of a hydroxyl group in C-5 was impossible in NOESY experiment. Therefore, the unambiguous determination of the absolute stereochemistry for the hydroxyl group in C-5 of **3** should be the subject of further study, such as CD analysis or X-ray crystallography.

The molecular formula of **10** was $\text{C}_{15}\text{H}_{20}\text{O}_4$ (MW, 264), as established by a pseudomolecular ion peak at m/z 287.1259 [$\text{M}+\text{Na}$] $^+$ (calculated

Fig. 3. Structures of isolated compounds from the aerial part of *Artemisia scoparia* and important ^1H - ^1H COSY (bold lines) and heteronuclear multiple-bond correlation (arrows) correlations for **1**, **3**, and **10**. The absolute stereostructure is not yet determined.

for $\text{C}_{15}\text{H}_{20}\text{O}_4\text{Na}$, 0 mDa) in HRESI-MS (positive). The ^1H - and ^{13}C -NMR spectra of **10** were similar to those of eudesmanolides (**24**). The ^1H -NMR spectrum exhibited a singlet methyl proton signal at δ 0.80 (3H, s, H-14) and two oxygenated methine proton signals at δ 3.54 (1H, dd, $J=12.0$ and 5.2 Hz, H-1) and 4.03 (1H, dd, $J=12.0$, and 5.8 Hz, H-3). An oxygenated quaternary carbon signal at δ 44.3 (C-10) in the ^{13}C -NMR spectrum was confirmed by HSQC, suggesting that **10** was eudesmadien-12,6-olide coupled to two hydroxyl groups. The planar structure of **10** was assigned as 1,4-dihydroxyeudesma-4(15),11(13)-dien-12,6-olide by ^1H - ^1H COSY (indicated by bold lines in Fig. 3) and HMBC (indicated by arrows in Fig. 3). Correlations of H-1/H-5, H-1/H-3, and H-5/H-7 were observed in the NOESY spectrum, suggesting that H-1, H-5, H-7, and H-15 had the same configuration. In addition, correlations of H-6/H-14 were observed in the NOESY spectrum, suggesting that H-6 and H-14 had the same configuration, which were different from those of H-1, H-5, H-7, and H-15. The ^{13}C -NMR spectrum and optical density of **10** were in agreement with that of 1,4-dihydroxyeudesma-4(15),11(13)-dien-12,6 α -olide (ludovicin B), as reported previously (**24**). Consequently, the structure of **10** was unambiguously identified as ludovicin B (Fig. 3).

ACE inhibitory activity of isolated compounds

The ACE inhibitory

activities of the identified compounds (**1**–**10**) are shown in Fig. 4. The isolated compounds showed ACE inhibitory activities ($6.8 \pm 0.2\%$ – $86.7 \pm 0.1\%$), albeit lower than that of captopril (1 nM, $51.2 \pm 3.3\%$) (data not shown), which has been used as a drug (**25**). However, scopoletins (**7**–**9**) exhibited significantly ($p < 0.05$) higher ACE inhibitory activities than sesquiterpene lactones (**1**–**6** and **10**) and quercetin. Among the scopoletins, scopoletin (**7**, $86.7 \pm 0.1\%$) showed higher ACE activity than **8** ($65.1 \pm 2.4\%$) and **9** ($69.0 \pm 3.0\%$), which possesses methyl and methoxyl groups at the C-7 and C-8 positions of scopoletin, respectively. Sesquiterpene lactones (**1**–**6** and **10**) had relatively low ACE inhibitory activities. Interestingly, compounds **3** and **4**, which possess dihydroxyl and epoxy groups at the C-3 and C-4 positions, respectively, showed significantly higher ACE inhibitory activities than other sesquiterpene lactones (**1**, **2**, **5**, and **6**), and their activities were similar to that of quercetin. Isofraxidin 7-O- β -D-glucopyranoside, a glucoside of isofraxidin (**9**) identified in this study, has been reported to show ACE inhibitory activity (**14,26**). In addition, sesquiterpene lactones and scopoletins have been reported to show antioxidative and anti-inflammatory activities (**27–31**). We previously found that the aerial part of *A. scoparia* reduced plasma lipid peroxidation and protein carbonyl oxidation and suppressed tumor necrosis factor alpha and NF- κ B expression induced in spontaneously

Table 1. ^1H -NMR data of the isolated compounds **1–6** and **10** from the aerial part of *Artemisia scoparia*

Position	δ_{H} (rel. int., mult., J in Hz)						
	1 ^a	2 ^b	3 ^a	4 ^a	5 ^b	6 ^b	10 ^b
1	3.10 (1H, td, 8.5, 2.0)	3.04 (1H, m)	3.04 (1H, dd, 12.0, 4.8)	2.97 (1H, td, 11.0, 8.0)	3.01 (1H, dd, 6.8, 2.7)	4.97 (1H, dd, 11.4, 4.8)	3.54 (1H, dd, 12.0, 5.2)
2a	2.16 (2H, t, 8.5)	2.22 (1H, m)	2.66–2.62 (1H, m)	2.06 (1H, dd, 14.0, 7.5)	4.38 (1H, br. s)	2.44 (2H, m)	2.13 (1H, dd, 12.0, 10.2)
2b		2.01 (1H, m)	2.33 (1H, m)	1.81 (1H, dd, 14.0, 11.5)			1.51 (1H, td, 12.0, 12.0)
3	-	4.17 (1H, dd, 12.6, 7.2)	5.70 (1H, m)	3.37 (1H, s)	5.79 (1H, br. s)	5.15 (1H, dd, 10.8, 6.0)	4.03 (1H, dd, 12.0, 5.8)
4	2.30 (1H, d, 7.5)	-	-	-	-	-	-
5	2.31 (1H, m)	2.38 (1H, dd, 12.0, 12.0)	-	2.31 (1H, dd, 11.0, 8.0)	3.08 (1H, dd, 9.0, 9.0)	4.98 (1H, d, 10.4)	2.14 (1H, d, 10.8)
6	4.40 (1H, 8.8)	4.15 (1H, dd, 12.0, 9.0)	4.20 (1H, d, 8.4)	4.07 (1H, dd, 11.0, 9.0)	4.04 (1H, dd, 10.2, 9.0)	4.77 (1H, dd, 10.2, 8.4)	4.19 (1H, dd, 10.8, 10.8)
7	3.00 (1H, dddd, 8.0, 8.0, 3.0, 3.0)	2.86 (1H, m)	3.35 (1H, m)	2.87 (1H, m)	2.98 (1H, m)	2.68 (1H, m)	2.62 (1H, ddd, 10.8, 10.8, 3.0)
8a	2.35 (1H, m)	2.31 (1H, m)	2.30 (1H, m)	2.26 (2H, m)	2.56 (1H, ddd, 12.6, 5.1, 5.1)	2.15 (1H, m)	1.60 (1H, dd, 12.0, 12.0)
8b	1.48 (1H, m)	1.34 (1H, m)	2.27 (1H, m)		2.27 (1H, m)	1.74 (1H, m)	2.08 (1H, m)
9a	2.63 (1H, m)	2.70 (1H, ddd, 12.6, 3.6, 3.6)	2.52 (1H, m)	2.21 (1H, m)	2.33 (1H, m)	2.40 (1H, m)	2.05 (1H, ddd, 13.2, 3.0, 3.0)
9b	2.22 (1H, m)	2.00 (1H, m)	2.24 (1H, m)	1.53 (1H, m)	1.44 (1H, m)	2.18 (1H, m)	1.36 (1H, ddd, 13.2, 13.2, 4.8)
13a	6.30 (1H, d, 3.5)	6.16 (1H, d, 3.3)	6.24 (1H, d, 3.3)	6.20 (1H, d, 1.5)	6.15 (1H, d, 3.3)	6.20 (1H, d, 3.6)	5.23 (1H, d, 1.8)
13b	5.58 (1H, d, 3.5)	5.63 (1H, d, 3.3)	5.53 (1H, d, 3.3)	5.48 (1H, d, 1.5)	5.61 (1H, d, 3.3)	5.66 (1H, d, 3.6)	4.94 (1H, d, 1.8)
14a	5.02 (1H, br. s)	5.08 (1H, br. s)	4.97 (1H, br. s)	4.95 (1H, br. s)	4.92 (1H, br. s)	1.71 (3H, s)	0.80 (3H, s)
14b	4.69 (1H, br. s)	5.02 (1H, br. s)	4.87 (1H, br. s)	4.86 (1H, d, 1.5)	4.77 (1H, br. s)		
15	1.28 (3H, d, 6.5)	1.30 (3H, s)	1.83 (3H, s)	1.62 (3H, s)	1.91 (3H, s)	1.50 (3H, s)	6.01 (1H, d, 3.0) 5.51 (1H, d, 3.0)

^aCDCl₃ and ^bCD₃OD were used as NMR solvents.**Table 2.** ^{13}C -NMR data of isolated compounds **1–6** and **10** from the aerial part of *Artemisia scoparia*

Position	δ_{C}						
	1 ^a	2 ^b	3 ^a	4 ^a	5 ^b	6 ^b	10 ^b
1	39.8	40.7	56.0	44.8	54.4	124.9	77.0
2	44.0 ^c	36.3	35.6	33.0	90.3	33.1	41.4
3	219.2	67.5	129.5	63.2	128.4	81.0	70.6
4	50.6	81.0	141.2	65.8	148.5	140.3	147.7
5	47.1	48.7	85.2	50.8	56.2	127.2	51.4
6	88.8	84.9	86.0	80.5	87.1	83.0	81.2
7	44.0 ^c	40.5	39.7	44.0	46.6	51.3	51.0
8	31.8	32.9	31.2	28.5	32.6	29.2	22.5
9	38.7	40.5	35.1	29.1	37.5	42.1	37.1
10	148.7	148.7	147.2	146.0	149.4	140.6	44.3
11	138.7	140.9	139.4	139.5	141.3	141.8	141.3
12	169.9	172.2	170.2	169.7	172.3	172.7	172.9
13	121.3	121.2	120.8	120.2	121.1	120.7	117.5
14	113.1	113.7	113.0	115.3	114.3	16.7	12.2
15	14.2	17.9	13.7	18.5	17.3	12.7	107.1

^aCDCl₃ and ^bCD₃OD were used as NMR solvents.^cThe signals of C-2 and C-7 overlapped.

Fig. 4. ACE inhibitory activity of compounds isolated from the aerial parts of *Artemisia scoparia*. The reaction solution comprising 0.1 M sodium borate buffer (pH 8.3) contains 0.3 M NaCl and 5 mM HHL solution as a substrate, and rabbit lung acetone powder solution as an ACE source was incubated at 37°C for 1 h. The absorbance of released hippuric acid was determined at 228 nm. The isolated compounds were assayed at a concentration of 1 mM. Quercetin (Q) was used as a positive control. Values are expressed as means $\pm$ SD ($n=3$). Different letters indicate a significant difference among compounds at $p<0.05$.

hypertensive rats (14). Therefore, the sesquiterpene lactones and scopoletins identified in this study might be partially responsible for the antioxidant and anti-inflammatory activities of *A. scoparia*.

In this study, 10 compounds (1–10) were isolated from the aerial parts of *A. scoparia* (Fig. 3) and seven sesquiterpene lactones (1–6 and 10) and three scopoletins (7–9) were identified. To our knowledge, compound 3 is newly identified in nature. Scopoletins, (7–9) including the glucoside of 9, have been previously found in *A. scoparia* (1,14). In addition, the occurrence of sesquiterpene lactones, including 4 and 10 in *Artemisia* species, has been investigated (32–35). Seven sesquiterpene lactones (1–3 and 10) are firstly isolated from this plant. We previously reported that *A. scoparia* attenuated the increment of blood pressure in spontaneously hypertensive rats by reducing ACE activity and angiotensin II production. Seven ACE inhibitors, including one new phenolic glycoside, were isolated from this plant (14). Furthermore, the 10 compounds identified in this study might contribute to the antihypertensive effects of *A. scoparia*. These results warrant further studies of the chemical constituents and biological effects of *A. scoparia*.

Acknowledgments This study was supported by grant 20130290 to the Solar Salt Research Center of Mokpo, National University, from the Ministry of Oceans and Fisheries, Korea.

Disclosure The authors declare no conflict of interest.

References

- Chen YL, Huang HC, Weng YI, Yu YJ, Lee YT. Morphological evidence for the antiatherogenic effect of scoparone in hyperlipidemic diabetic rabbit.

- Cardiovasc. Res. 28: 1679–1685 (1994)
- Yahagi T, Yakura N, Matsuzaki K, Kitanaka S. Inhibitory effect of chemical constituents from *Artemisia scoparia* Waldst. et Kit. on triglyceride accumulation in 3T3-L1 cells and nitric oxide production in Raw 264.7 cells. J. Nat. Med. 68: 414–420 (2014)
- Oh HK, Beon MS, Lee MW, Whang BC. Ecological motif on the salt-water plants of brackish area in Buandam. Korean J. Environ. Eco. 20: 311–318 (2006)
- Gillani AUH, Janbaz KH. Protective effect of *Artemisia scoparia* extract against acetaminophen-induced hepatotoxicity. Gen. Pharmacol. 24: 1455–1458 (1993)
- Lee YM, Hsiao G, Chang JW, Sheu JR, Yen MH. Scoparone inhibits tissue factor expression in lipopolysaccharide-activated human umbilical vein endothelial cells. J. Biomed. Sci. 10: 518–525 (2003)
- Pan SL, Huang YW, Guh JH, Chang YL, Peng CY, Teng CM. Esculetin inhibits rasmad-mediated cell proliferation and attenuates vascular restenosis following angioplasty in rats. Biochem. Pharmacol. 65: 1897–1905 (2003)
- Cha JD, Jeong MR, Jeong SI, Moon SE, Kim JY, Kii BS, Song YH. Chemical composition and antimicrobial activity of the essential oils of *Artemisia scoparia* and *A. capillaris*. Planta Med. 71: 186–190 (2005)
- Yoon WJ, Lee JA, Kim JY, Oh DJ, Jung YH, Lee WJ, Park SY. Antioxidant activities and anti-inflammatory effects on *Artemisia scoparia*. Korean J. Pharmacogn. 37: 235–240 (2006)
- Singh HP, Kaur S, Mittal S, Batish DR, Kohli RK. *In vitro* screening of essential oil from young and mature leaves of *Artemisia scoparia* compared to its major constituents for free radical scavenging activity. Food Chem. Toxicol. 48: 1040–1044 (2010)
- Habib M, Waheed L. Evaluation of anti-nociceptive, anti-inflammatory and antipyretic activities of *Artemisia scoparia* hydromethanolic extract. J. Ethnopharmacol. 145: 18–24 (2013)
- Khan K, Fatima H, Taqi MM, Zia M, ur-Rehman T, Mirza B, Haq I. Phytochemical and *in vitro* biological evaluation of *Artemisia scoparia* Waldst. & Kit for enhanced extraction of commercially significant bioactive compounds. J. Appl. Res. Med. Aromat. Plants 2: 77–86 (2015)
- Wang ZQ, Zhang XH, Yu Y, Tipton RC, Raskin I, Ribnick D, Johnson W, Cefalu WT. *Artemisia scoparia* extract attenuates non-alcoholic fatty liver disease in diet-induced obesity mice by enhancing hepatic insulin and AMPK signaling independently of FGF21 pathway. Metabolism 62: 1239–1249 (2013)
- Jamwal KS, Sharma ML, Chandhoke N, Ghatak BJ. Pharmacological action of 6,7-dimethoxycoumarin (scoparone) isolated from *Artemisia scoparia*, Waldst & Kit. Indian J. Med. Res. 60: 763–771 (1972)
- Cho JY, Park KH, Hwang DY, Lily J, Park YK, Kim SY, Kim HR, Moon JH, Ham KS. Antihypertensive effects of *Artemisia scoparia* Waldst in spontaneously hypertensive rats and identification of angiotensin I converting enzyme inhibitors. Molecules 20: 19789–19804 (2015)
- Cushman D, Cheung H. Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. Biochem. Pharmacol. 20: 1637–1648 (1971)
- Cho JY, Yang X, Park KH, Park HJ, Park SY, Moon JH, Ham KS. Isolation and identification of antioxidative compounds from *Suaeda japonica* and their antioxidative activities. Food Sci. Biotechnol. 22: 1547–1557 (2013)
- Ma CM, Ke W, Sun ZL, Peng JY, Li ZX, Zhou X, Fan GR, Huang CG. Large-scale isolation and purification of scoparone from *Herba artemisiae scopariae* by high-speed counter-current chromatography. Chromatographia 64: 83–87 (2006)
- Liu Z, Li S, Han N, Sun D, Gao Y, Yin Y. Studies on the chemical constituents of the vines of *Streptocaulon juvenas* (Lour) Merr. Asian J. Tradit. Med. 3: 193–198 (2008)
- Jung CM, Kwon HC, Choi SZ, Lee JH, Lee DJ, Ryu SN, Lee KR. Phytochemical constituents of *Ainsliaea acerifolia*. Korean J. Pharmacogn. 31: 125–129 (2000)
- Sigstad EE, Catalan CAN, Gutierrez AB, Diaz JG, Goedken VL, Herz W. Guaianolides and germacranolides from *Stevia grisebachiana*. Phytochemistry 30: 1933–1940 (1991)
- Adekenov SM, Mukhametzhanov MN, Kagarlitskii AD, Turmukhambetov AZ. A chemical investigation of *Achillea nobilis*. Chem. Nat. Compd. 20: 568–571 (1984)
- Hilmi F, Stricher O, Heilmann J. New cytotoxic sesquiterpene lactones from *Varionia saharae*. Planta Med. 69: 462–464 (2003)
- Hajdú Z, Zupkó I, Réthy B, Forgo P, Hohmann J. Bioactivity-guided isolation of cytotoxic sesquiterpenes and flavonoids from *Anthemis ruthenica*. Planta Med. 6: 94–96 (2010)
- Hu J, Zhu Q, Jia, Z. Assignment of the ^1H and ^{13}C -NMR spectra of ludovicin B by two-dimensional NMR techniques. Bull. Soc. Chim. Belg. 106: 141–146 (1997)
- Salveti A, Pedrinelli R, Arzilli F, Abdel-Haq B, Magagna A, Lucarina A, Graziadei L, Nuccorini A, Taddei S. Angiotensin-converting enzyme inhibitors in hypertension: A review. Int. J. Clin. Pharmacol. Res. 5: 429–438 (1985)
- Simaratanamongkol A, Umehara K, Noguchi H, Panichayupakaranant P. Identification of a new angiotensin-converting enzyme (ACE) inhibitor from Thai edible plants. Food Chem. 165: 92–97 (2014)
- Seifried HE, Anderson DE, Fisher EI, Milner JA. A review of the interaction

- among dietary antioxidants and reactive oxygen species. *J. Nutr. Biochem.* 11: 567-579 (2007)
28. González R, Ballester I, López-Posadasa R, Suárezb MD, Zarzuelob A, Martínez-Augustinb O, Sánchez De Medinaa F. Effects of flavonoids and other polyphenols on inflammation. *Crit. Rev. Food Sci. Nutr.* 51: 331-362 (2011)
 29. Merfort I. Perspectives on sesquiterpene lactones in inflammation and cancer. *Curr. Drug Targets* 12: 1560-1573 (2011)
 30. Gach K, Dlugosz A, Janecka A. The role of oxidative stress in anticancer activity of sesquiterpene lactones. *N-S Arch. Pharmacol.* 388: 477-486 (2015)
 31. Ivanescu B, Miron A, Corciova A. Sesquiterpene lactones from *Artemisia* genus: Biological activities and methods of analysis. *J. Anal. Methods Chem.* 2015: Article ID 247685 (2015)
 32. Sanchez-Viesca F, Romo J. Estafiatin, a new sesquiterpene lactone isolated from *Artemisia mexicana* (Willd). *Tetrahedron* 19: 1285-1291 (1963)
 33. Lee KH, Geissman TA. Sesquiterpene lactones of *Artemisia* constituents of *A. ludoviciana* ssp. *Mexicana*. *Phytochemistry* 9: 403-408 (1970)
 34. Hu J, Zhu Q, Bai S, Jia Z. New eudesmane sesquiterpene and other constituents from *Artemisia mongolica*. *Planta Med.* 62: 477-478 (1996)
 35. Ahmed AA, El-Moghazy SA, El-Shanawany MA, Abdel-Ghani HF, Karchesy J, Sturtz G, Dalley K, Pare PW. Polyol monoterpenes and sesquiterpene lactones from the Pacific Northwest plant *Artemisia suksdorfii*. *J. Nat. Prod.* 67: 1705-1710 (2004)