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Coumarins from the aerial parts of *Artemisia iwayomogi* Kitamura

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Abstract *Artemisia iwayomogi* (Compositae), a perennial mugwort, is native to Korea and widely distributed in Japan, Russia, and China. *A. iwayomogi* and *A. capillaris* are similar in morphology and pharmacological activity and have been used for the same medicinal purposes in Korea. While various ingredients such as coumarins and flavonoids and their activity studies have been reported for *A. capillaris*, few studies have been conducted on the pharmacologically active components of *A. iwayomogi*. In Korea, *A. capillaris* is not economical because only young leaves are used as a medicinal material. Because of this, *A. iwayomogi* is frequently used in Korea, indicating the need to study its pharmacologically active components. Therefore, a phytochemical study was initiated to isolate active compounds from the aerial parts of *A. iwayomogi*. Finally, four coumarins, umbelliferone (**1**), esculetin (**2**), grevillone (**3**), and scoparone (**4**) were isolated for the first time from the aerial parts of *A. iwayomogi* in this study.

Keywords *Artemisia iwayomogi* · Coumarin · Nuclear magnetic resonance · Preparative MPLC

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Introduction

Artemisia iwayomogi Kitamura (Compositae) is a perennial aromatic herb with yellow flowers. It is distributed in Korea, Japan, Sakhalin, the Kuril Islands in Russia, and Manchuria in China [1-3]. More than 30 species in this genus are found in Korea [4]. Among them, *A. iwayomogi* and *A. capillaris* are similar in terms of external appearance and pharmacological use. The aerial parts of *A. iwayomogi* and *A. capillaris* have been used in traditional medicine with the Korean name “Injin” [4]. *A. capillaris* is a common perennial herb and has been used for treatment of hepatitis, carbuncle, cholecystitis, and jaundice [2,3]. The major active components of *A. capillaris* are coumarins and flavonoids [5]. Though many phytochemical and pharmacological studies on *A. capillaris* have been carried out, only a few studies have been conducted on the pharmacologically active components of *A. iwayomogi*. Therefore, this study was initiated to isolate active compounds from the aerial parts of *A. iwayomogi*.

Coumarins, are 1,2-benzopyrones or 2H-1-benzopyran-2-ones, that consist of benzene rings joined by a α-pyrone ring and conjugated system with rich electron and good charge-transport properties [68]. They were synthesized via the phenylpropanoid pathway and have diverse structural modifications [9]. Coumarin is an oxygen heterocycle that occurs either in free or combined form with glucose. They have received attention for their diverse bioactivities. Coumarins were reported to act as competitive inhibitors of vitamin K but also show selective cytotoxicity on tumor cells [10]. They also regulate the immune response, cell growth, and differentiation [11]. In addition, some of its derivatives have been used as aroma agent for enhancing sense of tobaccos, alcoholic drinks, and perfumes [12,13]. Hence, the EtOAc fraction of *A. iwayomogi* and isolated coumarins may be valuable for use as additives in food, cosmetic, and drug industries.

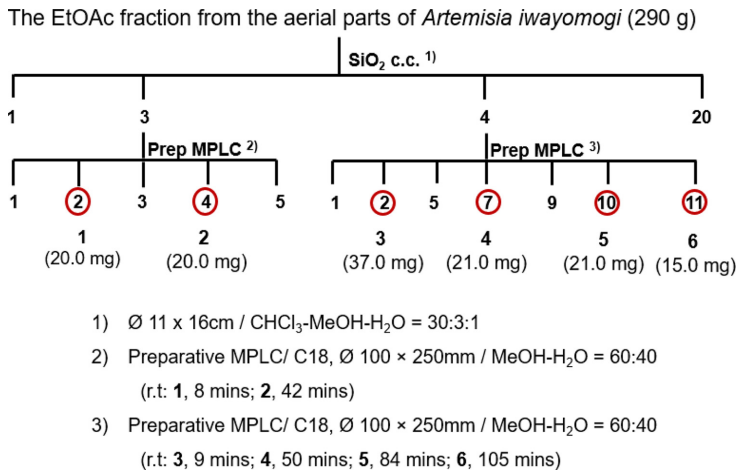

Fig. 1 Isolation procedure of coumarins from the aerial parts of *Artemisia iwayomogi*

Materials and Methods

Plant materials

The aerial parts of *Artemisia iwayomogi* were provided by RDA, Eumseong, Korea, in 2019 and identified by Dr. Jin Tae Jung, RDA, Eumseong, Korea. A voucher specimen has been stored in Natural Products Chemistry Laboratory, Kyung Hee University, Yongin, Korea.

General experimental procedures

The materials and equipment we used for the isolation and structure determination of constituents are described in a previous study [14].

Isolation of the coumarins from the aerial parts of *A. iwayomogi*

The dried aerials parts of *A. iwayomogi* (4.5 kg) were soaked in 80% aqueous MeOH (4.5 L×3) at room temperature for 24 h. After filtration, the extract was concentrated to afford 1.07 kg of residue. The obtained concentrate was suspended in H_2O (4.2 L) and sequentially extracted with EtOAc (4 L×3) and *n*-BuOH (3.6 L×3), which were concentrated to obtain EtOAc (AIE, 290 g), *n*-BuOH (AIB, 220 g), and H_2O (AIW, 560 g) fractions, respectively. The isolation procedures of compounds **1**-**6** from the aerial parts of *A. iwayomogi* are presented in Fig. 1.

Umbelliferone (**1**) Colorless needles; positive FAB-MS *m/z* 163 $[M+H]^+$; IR (KBr, *v*, cm^{-1}) 3077 (-OH), 1677 (γ-lactone C=O), 1562 and 1507 (aromatic benzene ring); ^{1}H-NMR (600 MHz, δ_H) (PMR) and ^{13}C-NMR (150 MHz, δ_C) (CMR) (C_5D_5N) see Tables 1 and 2.

Esculetin (**2**) Colorless needles; positive FAB-MS m/z 179 $[M+H]^+$; IR (KBr, *v*, cm^{-1}) 3413 (-OH), 1656 (γ-lactone C=O), 1567 (aromatic benzene ring); PMR and CMR (CD_3OD) see Tables 1 and 2.

Grevillone (**3**) Colorless needles; positive FAB-MS m/z 163 $[M+H]^+$; IR (KBr, *v*, cm^{-1}) 3539 (-OH), 1654 (ã-lactone C=O),

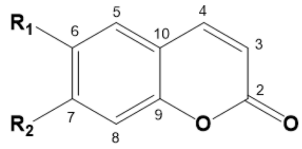

Fig. 2 Chemical structures of coumarins from the aerial parts of *Artemisia iwayomogi*

1574 and 1514 (aromatic benzene ring); PMR and CMR (C_5D_5N) see Tables 1 and 2.

Scoparone (**4**) Colorless needles; positive FAB-MS m/z 207 $[M+H]^+$; IR (KBr, *v*, cm^{-1}) 1648 (γ-lactone C=O), 1537 (aromatic); PMR and CMR ($CDCl_3$) see Tables 1 and 2.

Scopoletin (**5**) Yellow needles; positive FAB-MS m/z 193 $[M+H]^+$; IR (KBr, *v*, cm^{-1}) 3373 (-OH), 1648 (γ-lactone C=O), 1542 and 1510 (aromatic benzene ring); PMR and CMR (C_5D_5N) see Tables 1 and 2.

Isoscopoletin (**6**) Colorless needles; positive FAB-MS m/z 193 $[M + H]^+$; IR (KBr, *v*, cm^{-1}) 3285 (-OH), 1627 (γ-lactone C=O), 1560 and 1508 (aromatic benzene ring); PMR and CMR (C_5D_5N) see Tables 1 and 2.

Results and Discussion

The aerial parts of *A. iwayomogi* were extracted in aqueous MeOH, and the obtained extracts were partitioned using EtOAc, *n*-BuOH, and water. Repeated silica gel column chromatography and prep MPLC of AIE fraction yielded six coumarins.

Compound **1**, a colorless needle, was showed a pink color on

Table 1 ^{13}C-NMR data of coumarins from the aerials parts of *Artemisia iwayomogi* (150 MHz, δ_C)

No of C	1*	2	3	4	5	6
2	162.54	162.16	170.23	161.64	161.93	162.48
3	114.45	112.37	117.05	114.55	113.97	114.86
4	144.36	145.17	146.67	144.24	143.85	145.26
5	129.87	112.04	115.86	108.46	112.57	112.13
6	111.63	142.93	150.71	147.78	148.68	147.19
7	161.37	148.85	127.53	151.92	145.24	156.69
8	102.49	103.15	117.16	100.61	100.05	105.37
9	156.92	151.16	147.94	150.82	152.64	154.83
10	111.43	111.48	127.65	113.33	112.73	111.39
6-OMe	-	-	-	56.74	56.59	-
7-OMe	-	-	-	56.74	-	57.26

*Compounds **1**, **3**, **5**, and **6** in C_5D_5N; compounds **2** and **4** in CD_3OD

Table 2 ^{1}H-NMR data of coumarins from the aerials parts of *Artemisia iwayomogi* (600 MHz, δ_H, coupling pattern, *J* in Hz)

No of H	1*	2	3	4	5	6
3	7.69, d, 9.0	7.92, d, 9.0	8.19, d, 9.0	7.64, d, 9.0	7.69, d, 9.0	7.7, d, 9.0
4	6.3, d, 9.0	6.13, d, 9.0	6.81, d, 9.0	6.29, d, 9.0	6.39, d, 9.0	6.31, d, 9.0
5	7.43, d, 9.0	6.69, s	7.66, s	6.87, s	7.25, s	7.14, s
6	7.08, dd, 9.0, 2.0	-	-	-	-	-
7	-	-	7.25, dd, 9.0, 2.0	-	-	-
8	7.05, d, 2.0	6.71, s	7.21, d, 9.0	6.85, s	6.69, s	7.07, s
6-OMe	-	-	-	3.93, s	3.91, s	-
7-OMe	-	-	-	3.95, s	-	3.80, s

*Compounds **1**, **3**, **5**, and **6** in C_5D_5N; compounds **2** and **4** in CD_3OD

TLC by 10% sulfuric acid spraying and alcohol lamp heating. It showed IR absorbance bands (cm^{-1}) due to hydroxyl (3077), conjugated ketone (1677), and aromatic (1562 and 1507) groups. The molecular weight (MW) was determined to be 162 Da based on a detected molecular ion peak *m/z* 163 $[M + H]^+$ in the positive FAB-MS spectrum. PMR spectrum (C_5D_5N) exhibited the signals of three olefin methines δ_H 7.43 (1H, d, *J*=9.0 Hz, H-5), δ_H 7.08 (1H, dd, *J*=9.0, 2.0 Hz, H6), δ_H 7.05 (1H, d, *J*=2.0 Hz, H8) due to a 1,2,4-trisubstituted benzene ring. Moreover, the signals of two olefin methines δ_H 7.69 (1H, d, *J*=9.0 Hz, H3) and δ_H 6.30 (1H, d, *J*=9.0 Hz, H4) were observed with vicinal-coupling. CMR (C_5D_5N) spectrum showed 9 carbon signals. In a low magnetic field, the carbon signals of one ester (δ_C 162.54, C2), two oxygenated olefinic quaternary carbons (δ_C 156.92, C9; δ_C 161.37, C7), and five olefinic methine carbons (δ_C 144.36, C4; δ_C 129.87, C5; δ_C 114.45, C3; δ_C 111.63, C6; δ_C 102.49, C8) were observed. The above described PMR and CMR signals suggested compound **1** to be a coumarin with one hydroxy group. The HMBC spectrum showed the cross-peaks the oxygenated aromatic quaternary carbon signal (δ_C 161.37, C7) with three aromatic methine proton signals δ_H 7.43 (H5), δ_H 7.08 (H6), and δ_H 7.05 (H8), respectively, which confirmed the hydroxy group at C7. Therefore, compound **1** was identified as a 7-hydroxychromen-2-one, umbelliferone. The chemical shifts of PMR and CMR were confirmed by comparison with literature data [15].

Compound **2**, a colorless needle, was showed a pink color on TLC by 10% sulfuric acid spraying and alcohol lamp heating. It showed IR absorbance bands (cm^{-1}) due to hydroxyl (3413), conjugated ketone (1656), and aromatic (1567) groups. The MW was determined to be 178 Da, which was 16 Da larger than that of compound **1**, based on a detected molecular ion peak *m/z* 179 $[M+H]^+$ in the positive FAB-MS spectrum, indicating an additional hydroxy group in compound **2**. PMR and CMR (CD_3OD) spectra of compound **2** were very similar to those of compound **1**, with the exception of the presence of one additional hydroxy group. An olefinic carbon (C6), which was detected at δ_C 111.66 in compound **1**, was detected at a lower magnetic field, δ_C 142.93, in compound **2**. Therefore, compound **2** was a 6,7-dihydroxychromen-2-one, esculetin, a hydroxylated coumarin of umbelliferone.

Compound **3**, a colorless needle, was showed a pink color on TLC by 10% sulfuric acid spraying and alcohol lamp heating to. It showed IR absorbance bands (cm^{-1}) due to hydroxyl (3539), conjugated ketone (1654), and aromatic (1574 and 1514) groups. The MW was determined to be 162 based on a detected molecular ion peak *m/z* 163 $[M+H]^+$ in the positive FAB-MS spectrum.

PMR and CMR (C_5D_5N) spectra of compound **3** were nearly identical to those of compound **1** but showed a different hydroxyl group position. The HMBC spectrum showed correlation of the oxygenated olefinic quaternary carbon signal δ_C 111.63 (C6) with three olefinic methine proton signals at δ_H 7.66 (H5), δ_H 7.25 (H7), and δ_H 7.21 (H8), which confirmed the hydroxy group at C6. Therefore, compound **3** was identified to be a 7-hydroxychromen-2-one, grevillone.

Compound **4**, a colorless needle, was showed a pink color on TLC by 10% sulfuric acid spraying and alcohol lamp heating. It showed IR absorbance bands (cm^{-1}) due to conjugated ketone (1648) and aromatic (1537) groups. The MW was determined to be 206 Da based on a detected molecular ion peak *m/z* 207 $[M+H]^+$ in the positive FAB-MS spectrum, which was 28 Da larger than compound **2**, suggesting transformation of two hydroxy groups to two methoxy groups. The PMR and CMR ($CDCl_3$) spectra exhibited two methoxy groups at δ_H 3.93 (6OMe), δ_H 3.95 (7OMe), and δ_C 56.74 (6OMe, 7OMe). In the HMBC spectrum, the signals of two methoxy protons δ_H 3.93 (6OMe) and δ_H 3.95 (7OMe) showed cross peaks with two oxygenated olefinic quaternary carbon signals at δ_C 151.92 (C7) and δ_C 147.78 (C6), respectively. Therefore, compound **4** was identified as a 6,7-dimethoxychromen-2-one, scoparone.

Compound **5**, a colorless needle, was showed a pink color on TLC by 10% sulfuric acid spraying and alcohol lamp heating. It showed IR absorbance bands (cm^{-1}) due to hydroxyl (3373), conjugated ketone (1648), and aromatic (1542 and 1510) groups. The MW was determined to be 192 Da based on a detected molecular ion peak *m/z* 193 $[M+H]^+$ in the positive FAB-MS spectrum, which was 14 Da larger than compound **2**, 178 Da, indicating one hydroxy group was transformed to a methoxy group. The PMR and CMR (C_5D_5N) spectra were almost same as those of compound **5** with the exception of one additional signal due to a methoxy group δ_H 3.91 (6OMe) and δ_C 56.59 (6OMe). Therefore, compound **5** was identified to be a 7-hydroxy-6-methoxychromen-2-one, scopoletin.

Compound **6**, a colorless needle, was showed a pink color on TLC by 10% sulfuric acid spraying and alcohol lamp heating. It showed absorbance bands (cm^{-1}) due to hydroxyl (3285), conjugated ketone (1627), and aromatic (1560 and 1508) groups. The MW was determined to be 192 Da based on a detected molecular ion peak *m/z* 193 $[M+H]^+$ in the positive FAB-MS spectrum. The PMR and CMR (C_5D_5N) spectra were similar to those of compound **5** with the exception one methoxy and one hydroxy group position. The position of the methoxy group was determined to be C7 from the cross peak between the methoxy proton signal δ_H 3.80 (7OMe) and the oxygenated olefinic methine carbon signal δ_C 156.69 (C7). Finally, compound **6** was identified as a 6-hydroxy-7-methoxychromen-2-one, isoscopoletin.

In conclusion, this study was initiated to search for active compounds from the aerial parts of *A. iwayomogi*. Six coumarins were isolated through repeated column chromatography using SiO_2 resins and prep-MPLC and identified on the basis of spectroscopic data of NMR, IR, UV, and MS. Especially, four coumarins, umbelliferone (**1**), esculetin (**2**), grevillone (**3**), and scoparone (**4**) were isolated for the first time from the aerial parts of *A. iwayomogi* in this study. These results indicate that the aerial parts of *A. iwayomogi* could be used as potential pharmacological agents.

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