Arch Pharm Res Vol 25, No 3, 285-288, 2002

Archives of Pharmacal Research
http://apr.psk.or.kr

Phytochemical Constituents from the Herba of *Artemisia apiacea*

Sanghyun Lee, Kyoung Soon Kim, Ji Myun Jang, Youmie Park, Yang Bae Kim, and Bak-Kwang Kim
College of Pharmacy, Seoul National University, Seoul 151-742, Korea

(Received April 14, 2002)

Four compounds of a coumarin, a steroid and two flavonoids were isolated from the herba of *Artemisia apiacea* by open column chromatography. Their structures were elucidated as artemicapin C, apigenin, daucosterol and cacticin by chemical and spectroscopic analysis. This is the first report of the isolation of these compounds from this plant.

Key words: *Artemisia apiacea*, Compositae, Artemicapin C, Apigenin, Daucosterol, Cacticin

INTRODUCTION

Artemisia species spreads widely in nature. *Artemisia* species are genus of the family Compositae consisting of more than 350 species. *A. apiacea* is distributed at wasteland and river beaches of Korea, Japan and China. *A. apiacea* has been used as traditional medicine to treat eczema and jaundice (Yook, 1989).

The compounds isolated from *Artemisia* species such as terpenoids, flavonoids, coumarins, acetylenes, caffeoylquinic acids and sterols were shown to be anti-malarial, anti-viral, anti-tumor, anti-pyretic, anti-hemorrhagic, anti-coagulant, anti-anginal, anti-oxidant, anti-hepatitis, anti-ulcerogenic, anti-spasmodic and anti-complementary activities (Tan *et al.*, 1998).

Literature survey of *A. apiacea* revealed that no phytochemical and pharmacological studies of polar fractions have been performed. To date, investigations on the compounds of *A. apiacea* have revealed the presence of campesterol, stigmasterol, β-sitosterol, 7-methoxycoumarin, 7,8-dimethoxycoumarin and 7,8-methylenedioxycoumarin from the flower heads (Shimomura *et al.*, 1979), scopoletin, protocatechualdehyde and ethyl and methyl caffeates from the stems and leaves (Shimomura *et al.*, 1980a), daphnetin, 7-hydroxy-8-methoxycoumarin and 7-isopentenyloxy-8-methoxycoumarin from flower heads (Shimomura *et al.*, 1980b) and volatile constituents like α-pinene and artemisia ketone from the roots (Yano, 1970; Kim and Jang, 1994).

In the previous paper, *A. apiacea* was shown to be hair-growth activity (Kim *et al.*, 1999).

The chromatographic separation of the *n*-BuOH soluble fraction from this plant led to the isolation of a coumarin **1**, a steroid **3** and two flavonoids **2** and **4**. This paper describes the isolation and structural determination of these compounds.

MATERIALS AND METHOD

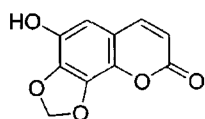
Instruments and reagents

Silica gel 60 (MERCK Co., 0.063-0.200 mm) was used for open column chromatography. Silica gel plates (MERCK Co., Kieselgel 60 F_{254}) were used for TLC. Spots were detected by spraying with 20% H_2SO_4 in MeOH and heating. IR spectra were recorded with JASCO FT/IR-300E instrument on KBr disc. ^{1}H- and ^{13}C-NMR spectra were recorded with BRUKER AVANCE 400 NMR spectrometer in DMSO-d_6 or pyridine using TMS as internal standard. Chemical shifts were reported in parts per million (δ), and coupling constants (*J*) were expressed in hertz. MS spectra were measured with JEOL JMS-AX505WA mass spectrometer. Other reagents were commercial grade without purification.

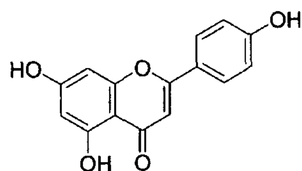
Plant materials

The herba of *Artemisia apiacea* Hance was purchased from the Kyungdong market, Korea in January 1999, and verified by Prof. Dae Suk Han, Seoul National University, Korea. A voucher specimen (Kim 99015) of this plant has been deposited at the Herbarium of College of Pharmacy,

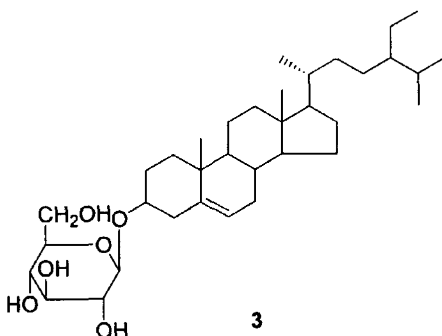
Correspondence to: Bak-Kwang Kim, College of Pharmacy, Seoul National University, Seoul 151-742, Korea
E-mail: kimbk2@snu.ac.kr (B-K. Kim)



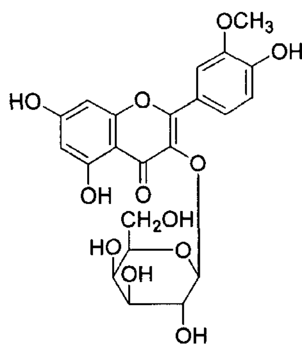
1



2



3



4

Seoul National University, Korea.

Extraction and isolation

The air-dried powdered herba of *A. apiacea* (5 kg) were extracted three times with MeOH under reflux. The resultant extracts were combined and concentrated under reduced pressure to afford 255 g of the residue. The MeOH extract was suspended in water and then fractionated successively with equal volumes of *n*-hexane, CH_2Cl_2, EtOAc and *n*-BuOH, leaving residual water-soluble fraction. Each fraction was evaporated *in vaccuo* to yield the residues of *n*-hexane soluble fraction (40 g), CH_2Cl_2 soluble fraction (38 g), EtOAc soluble fraction (56 g) and *n*-BuOH soluble fraction (30 g).

The resulting *n*-BuOH soluble fraction (30 g) was chromatographed on silica gel column eluting with a gradient of $CHCl_3$-MeOH to afford Fractions A-S. Compounds **1** (3 mg), **2** (7 mg), **3** (13 mg) and **4** (76 mg) were obtained from Fraction C (subfractions 28-29), Fraction E (subfractions 37-40), Fraction H (subfractions 46-50) and Fraction M (subfractions 63-70), respectively. Each Fraction was recrystallization with MeOH.

Compound 1 (6-hydroxy-7,8-methylenedioxy coumarin); ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 10.09 (1H, s, -OH), 7.93 (1H, d, *J*=9.6 Hz, H-4), 6.75 (1H, s, H-5), 6.27 (1H, d, *J*=9.6 Hz, H-3), 6.19 (2H, s, -OCH_2O-); ^{13}C-NMR (100 MHz, DMSO-d_6) δ_c (ppm): 159.7 (C-2), 145.2 (C-4), 139.2 (C-7), 138.7 (C-6), 134.6 (C-8), 132.2 (C-9), 113.6 (C-10), 112.9 (C-3), 109.2 (C-5), 103.5 (-OCH_2O-); IR ν_{max} (KBr) cm^{-1}: 3235 (OH), 1697 (C=O), 1625, 1584; EIMS *m/z* (rel. int. %): 206 $[M]^+$ (100), 178 (23.9), 148 (9.2), 120 (9.3), 108 (7.8), 92 (8.7), 79 (7.1).

Compound 2 (5,7,4'-trihydroxy flavone); ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 12.93 (1H, s, 5-OH), 10.90 (1H, br s, -OH), 10.40 (1H, s, -OH), 7.91 (2H, d, *J*=8.8 Hz, H-2' and 6'), 6.92 (2H, d, *J*=8.8 Hz, H-3' and 5'), 6.76 (1H, s, H-3), 6.48 (1H, d, *J*=2.2 Hz, H-8), 6.18 (1H, d, *J*=2.2 Hz, H-6); ^{13}C-NMR (100 MHz, DMSO-d_6) δ_c (ppm): 182.2 (C-4), 164.5 (C-7), 164.3 (C-2), 161.8 (C-4'), 161.6 (C-5), 157.8 (C-9), 128.9 (C-2' and 6'), 121.6 (C-1'), 116.4 (C-3' and 5'), 104.1 (C-10), 103.3 (C-3), 99.3 (C-6), 94.8 (C-8); IR ν_{max} (KBr) cm^{-1}: 3422 (OH), 1651 (C=O); EIMS *m/z* (rel. int. %): 270 $[M]^+$ (100), 242 (10.8), 153 (13.2), 152 (7.4), 121 (9.8), 118 (6.1), 69 (4.2).

Compound 3 (β-sitosterol 3-*O*-β-D-glucopyranoside); ^{1}H-NMR (400 MHz, Pyridine) δ_H (ppm): 5.35 (1H, br d, *J*= 5.1 Hz, H-6), 5.08 (1H, d, *J*=7.7 Hz, Glucosyl H-1'), 3.53 (2H, tt, *J*=5.1, 11.7 Hz, H-3), 1.01 (3H, d, *J*=6.6 Hz, 21-CH_3), 0.92 (3H, s, 19-CH_3), 0.84 (3H, t, *J*=7.6 Hz, 29-CH_3), 0.83 (3H, d, *J*=7.3 Hz, 26-CH_3), 0.81 (3H, d, *J*=6.8 Hz, 27-CH_3) 0.68 (3H, s, 18-CH_3); ^{13}C-NMR (100 MHz, Pyridine) δ_c (ppm): 141.1 (C-5), 121.9 (C-6), 102.7 (C-1'), 78.6 (C-3), 78.4 (C-3'), 78.3 (C-5'), 75.4 (C-2'), 71.9 (C-4'), 63.0 (C-6'), 57.0 (C-14), 56.4 (C-17), 50.5 (C-9), 46.2 (C-24), 42.6 (C-13), 40.1 (C-12), 39.5 (C-4), 37.6 (C-1), 37.0 (C-10), 36.4 (C-20), 34.3 (C-22), 32.3 (C-7), 32.2 (C-8), 30.3 (C-2), 29.7 (C-25), 28.6 (C-16), 26.6 (C-23), 24.6 (C-15), 23.5 (C-28), 21.4 (C-11), 20.0 (C-26), 19.5 (C-27), 19.3 (C-19), 19.1 (C-21), 12.2 (C-29), 12.1 (C-18); IR ν_{max} (KBr) cm^{-1}: 3422 (OH), 1095 (C-O); EIMS *m/z*: 414 $[\beta\text{-sitosterol}]^+$; FABMS *m/z*: 577 $[M+H]^+$.

Compound 4 (isorhamnetin 3-*O*-β-D-galactopyranoside); ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 12.62 (1H, s, 5-OH), 10.59 (1H, br s, -OH), 10.08 (1H, s, -OH), 8.02 (1H,

d, J=1.9 Hz, H-2'), 7.50 (1H, dd, J =8.4, 1.9 Hz, H-6'), 6.90 (1H, d, J=8.4 Hz, H-5'), 6.44 (1H, d, J=1.9 Hz, H-8), 6.21 (1H, d, J=1.9 Hz, H-6), 5.51 (1H, d, J=7.7 Hz, Galactosyl H-1"), 3.85 (3H, s, $-OCH_3$); ^{13}C-NMR (100 MHz, DMSO-d_6) δ_c (ppm): 177.8 (C-4), 164.7 (C-7), 161.6 (C-5), 156.8 (C-2), 156.6 (C-9), 149.8 (C-4'), 147.4 (C-3'), 133.5 (C-3), 122.3 (C-6'), 121.5 (C-1'), 115.5 (C-5'), 113.9 (C-2'), 104.4 (C-10), 102.0 (C-1"), 99.1 (C-6), 94.1 (C-8), 76.3 (C-5"), 73.5 (C-3"), 71.6 (C-2"), 68.3 (C-4"), 60.7 (C-6"), 56.4 (-OCH_3); IR ν_{max} (KBr) cm^{-1}: 3420 (OH), 1627 (C=O), 1093 (C-O); EIMS *m/z*: 316 [isorhamnetin]$^+$; FABMS *m/z*: 479 $[M+H]^+$.

Acid hydrolysis of 3 and 4

Each compound was refluxed with 5% H_2SO_4 in MeOH for 4 hr. Workup in the usual way, followed by crystallization afforded β-sitosterol and isorhamnetin from **3** and **4**, respectively.

RESULTS AND DISCUSSION

The chromatographic separation of the *n*-BuOH soluble fraction from *A. apiacea* herba led to the isolation of a coumarin: artemicapin C (**1**), a steroid: daucosterol (**3**) and two flavonoids: apigenin (**2**) and cacticin (**4**).

Compound **1** was obtained as white-yellow crystals from MeOH. The EIMS of **1** showed an $[M]^+$ ion at *m/z* 206 as a base peak. In the ^{1}H-NMR spectrum of **1**, the typical signals of coumarin H-3 and H-4 were observed at δ 6.27 (d, J=9.6 Hz) and δ 7.93 (d, J=9.6 Hz), respectively. The aromatic H-5 was observed at δ 6.75 (s). The singlet signal at δ 6.19 indicated methylenedioxy protons. Its ^{13}C-NMR spectrum of **1** showed C=O signal at δ 159.7 and methylenedioxy at δ 103.5. The IR spectrum of **1** showed adsorption bands for hydroxy at 3235 cm^{-1} and α,β-unsaturated C=O at 1697 cm^{-1}. Accordingly, the structure of **1** was elucidated as artemicapin C (6-hydroxy-7,8-methylenedioxy coumarin). It was newly reported as a new compound from the aerial part of *Artemisia capillaris* (Wu *et al.*, 2001).

Compound **2** was obtained as yellow crystals from MeOH. It responded positively to the Shinoda test. The EIMS of **2** showed an $[M]^+$ ion at *m/z* 270 as a base peak. The characteristic fragment ion peaks at *m/z* 153 and 121 showed the *retro* Diels Alder fragmentation of flavonoids (Markham, 1982). In the ^{1}H-NMR spectrum of **2**, the typical flavonoid signals were observed. The singlet of aromatic 5-OH was observed at δ 12.93, H-2',6' and H-3', 5' at δ 7.91 (d, J=8.8 Hz) and δ 6.92 (d, J=8.8 Hz), respectively. These doublet signals at δ 7.91 and δ 6.92 showed A_2B_2 splitting pattern of (B) ring due to a *para*-substituted benzene ring. The meta coupling of H-8 and H-6 of (A) ring showed at δ 6.48 (d, J=2.2 Hz) and δ 6.18 (d, J=2.2 Hz), respectively. The singlet signal of H-3 showed at δ 6.76. Its ^{13}C-NMR spectrum of **2** showed C=O at δ 182.2. The IR spectrum of **2** showed adsorption bands for hydroxy at 3422 cm^{-1} and α,β-unsaturated C=O at 1651 cm^{-1}. Accordingly, the structure of **2** was elucidated as apigenin (5,7,4'-trihydroxy flavone). Lee *et al.* (1994) and Lee *et al.* (1988) reported the isolation of apigenin. It reduced GABA-activated Cl^- currents in a dose-dependent fashion and locomotor activity by i.p. injection (Avallone *et al.*, 2000; Zanoli *et al.*, 2000).

Compound **3** was obtained as white crystals from MeOH. It responded positively to the Libermann-Burchard and the Molisch test. In the EIMS of **3**, aglycone peak showed at *m/z* 414. The FABMS of **3** showed $[M + H]^+$ peak at *m/z* 577 corresponding to the molecular formula $C_{35}H_{60}O_6$. In the ^{1}H-NMR spectrum of **3**, the angular methyl singlet signals of 18-Me and 19-Me at δ 0.68 and δ 0.92, and the doublet of 21-Me, 26-Me and 27-Me at δ 1.01, δ 0.83 and δ 0.81 were observed, respectively. The broad singlet at δ 5.34 showed H-6. The signals of δ 3.00-5.00 showed glycoside. The aglycone of **3** was β-sitosterol by acid hydrolysis. Due to the chemical shift of C-3 of β-sitosterol changed from δ 71.2 to δ 78.6 and the anomeric proton of glucose showed at δ 4.22 (d, J=7.7 Hz), glucose position was at C-3 (β-linkage) of aglycone. The IR spectrum of **3** showed adsorption bands for hydroxy at 3434 cm^{-1} and glycosidic C-O at 1073 and 1030 cm^{-1}. Accordingly, the structure of **3** was elucidated as daucosterol (β-sitosterol 3-*O*-β-D-glucopyranoside). Shang-jiang *et al.* (1986) reported the isolation of daucosterol from the roots of *Adenophora axilliflora*. And Chang *et al.* (1981) reported the ^{13}C-NMR assignment of β-sitosterol and β-sitosteryl-3-*O*-β-D-glucopyranoside. Daucosterol decreased vascular permeability and showed hemostatic effect (Sugiyama and Seki, 1991).

Compound **4** was obtained as yellow crystals from MeOH. It responded positively to the Shinoda and the Molisch test. In the EIMS of **4**, the aglycone peak showed at *m/z* 316. The characteristic fragment ion peaks at *m/z* 153 and 121 showed the *retro* Diels Alder fragmentation of flavonoids (Markham, 1982). The FABMS of **4** showed $[M + H]^+$ peak at *m/z* 479 corresponding to the molecular formula $C_{22}H_{22}O_{12}$. In the ^{1}H-NMR spectrum of **4**, the typical flavonoid signals were observed. The proton signals of δ 8.02 (d, J=1.9 Hz), δ 7.50 (dd, J=8.4, 1.9 Hz) and δ 6.90 (d, J=8.4 Hz) showed ABX splitting pattern of (B) ring. Accordingly, the position of OCH_3 was at C-3' of (B) ring by HMBC analysis. The singlet of aromatic 5-OH at δ 12.62 and the singlet of OCH_3 signal at δ 3.85 were observed, respectively. The proton signals at δ 3.00-5.00 showed glycoside. The aglycone of **4** was isorhamnetin by acid hydrolysis. Due to the chemical shift of C-3 of

isorhamnetin changed from δ 136.1 to δ 133.5 and the anomeric proton signal of galactose at δ 5.51 (d, J=7.7 Hz), galactose position was at C-3 (β-linkage) of aglycone. Its ^{13}C-NMR spectrum of **4** showed C=O at δ 177.8 and OCH_3 at δ 56.4. The carbon signal at δ 102.0 showed galactosyl C-1". The IR spectrum of **4** showed absorption bands for hydroxy at 3420 cm^{-1}. Accordingly, the structure of **4** was elucidated as cacticin (isorhamnetin 3-*O*-β-D-galactopyranoside). Jang *et al.* (1996) reported the isolation of cacticin from *Artemisia capillaris*. Kang *et al.* (1997) reported it from *Evodia rutaecarpa*. The extract of *Culcitium reflexum* showed antioxidant and photoprotective activity and their major compounds were flavonoids including cacticin (Aquino *et al.*, 2002).

These compounds, artemicapin C (**1**), apigenin (**2**), daucosterol (**3**) and cacticin (**4**) were isolated for the first time from the herba of *A. apiaceae*.

ACKNOWLEDGEMENTS

This work was partly supported by the Brain Korea 21 Project and Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Korea.

REFERENCES

Aquino, R., Morelli, S., Tomaino, A., Pellegrino, M., Saija, A., Grumetto, L., Puglia, C., Ventura, D., and Bonina, F., Antioxidant and photoprotective activity of a crude extract of *Culcitum reflexum* H. B. K. leaves and their major flavonoids. *J. Ethnopharmacol.*, 79, 183-191 (2002).

Avallone, R., Zanoli, P., Puia, G., Kleinschnitz, M.,Schreier, P., and Baraldi, M., Pharmacological profile of apigenin, a flavonoid isolated from *Matricaria chamomilla*. *Biochem. Pharmacol.*, 59, 1387-1394 (2000).

Chang I. M., Yun-Choi, H. S., and Yamasaki, K., Revision of ^{13}C NMR assignment of β-sitosterol and β-sitosteryl-3-O-β-D-glucopyranoside isolated from *Plantago asiatica* seed. *Kor. J. Pharmacogn.*, 12, 12-24 (1981).

Jang, M-H., Wu, T-S., and Su, J-D., Studies on antioxidative components from *Artemisia capillaris* Thunb. *Shipin Kexue*, 23, 594-607 (1996).

Kang, S. S., Um, B. H., Kim, J. S., and Ahn, B. T., Isolation of flavonoids from Evodiae fructus. *Kor. J. Pharmacogn.*, 28, 9-14 (1997).

Kim, K. S., Shim, S. H., Jang, J. M., Cheong, J. H., and Kim, B-K., A study on hair-growth activity of *Artemisia apiacea* Hance. *Yakhak Hoeji*, 43, 798-801 (1999).

Kim, O. C., and Jang, H. J., Volatile components of *Artemisia apiacea* Herba. *Hanguk Nonghwa Hakhoechi*, 37, 37-42 (1994).

Lee, H. B., Kwak, J. H., Zee, O. P., and Yoo, S. J., Flavonoids from *Circum rhinoceros*. *Arch. Pharm. Res.*, 17, 273-277 (1994).

Lee, Y. H., Lee, I. R., Woo, W. S., and Park, C. H., Flavonoids of *Elscholtzia cristata*. *Arch. Pharm. Res.*, 11, 247-249 (1988).

Markham, K. R., Techniques of flavonoid identification, Academic press, London, pp. 87-90, (1982).

Shang-jiang, D., Gariboldi, P., and Jommi, G., Constituents of Shashen (*Adenophora axilliflora*). *Planta Med.*, 49, 317-320 (1986).

Shimomura, H., Sashida, Y., and Ohshima, Y., Coumarins from *Artemisia apiacea*. *Phytochemistry*, 18, 1761-1762 (1979).

Shimomura, H., Sashida, Y., and Ohshima, Y., The chemical components of *Artemisia apiacea* Hance, More coumarins from the flower heads. *Chem. Pharm. Bull.*, 28, 347-348 (1980a).

Shimomura, H., Sashida, Y., Oshima, Y., Azuma, T., and Saitoh, M., The chemical components of *Artemisia apiacea* Hance, Components of stems and leaves. *Yakugaku Zasshi*, 100, 1164-1166 (1980b).

Sugiyama, M. and Seki, J., In vivo application of lipoproteins as drug carriers: pharmacological evaluation of sterylglucoside-lipoprotein complexes. *Targ. Diagn. Ther.* 5, 315-350 (1991).

Tan, R. X., Zheng, W. F., and Tang, H. Q., Biologically active substances from the genus *Artemisia*. *Planta Med.*, 64, 295-302 (1998).

Wu, T. S., Tsang, Z. J., Wu, P. L., Lin, F. W., Li, C. Y., Teng, C. M., and Lee, K. H., New constituents and antiplatelet aggregation and anti-HIV principles of *Artemisia capillaris*. *Bioorg. Med. Chem.*, 9, 77-83 (2001).

Yano, K., Mono- and sesqui-terpenes of the essential oils from *Artemisia japonica* and *Artemisia apiacea*. *Flavour Ind.*, 1, 328-330 (1970).

Yook, C., S., Coloured medicinal plants of Korea, p. 522, (1989).

Zanoli, P., Avallone, R., and Baraldi, M., Behavioral characterization of the flavonoids apigenin and chrysin. *Fitoterapia*, 71, S117-S123 (2000).