Arch Pharm Res Vol 26, No 11, 902-905, 2003

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

# Constituents from the Non-Polar Fraction of *Artemisia apiacea*

**Sanghyun Lee, Kyoung Soon Kim, Sang Hee Shim, You Mie Park, and Bak-Kwang Kim**
*College of Pharmacy, Seoul National University, Seoul 151-742, Korea*

*(Received August 16, 2003)*

Five compounds of terpenoids and coumarins were isolated from the non-polar fraction of *Artemisia apiacea* by open column chromatography. Their structures were elucidated as α-amyrin (**1**), β-amyrin (**2**), β-sitosterol (**3**), 5,6,7-trimethoxycoumarin (**4**) and 6-methoxy-7,8-methylenedioxycoumarin (**5**) by chemical and spectroscopic analysis. This is the first report of the isolation of α-amyrin, β-amyrin, 5,6,7-trimethoxycoumarin and 6-methoxy-7,8-methylenedioxycoumarin from this plant.

**Key words:** *Artemisia apiacea*, Compositae, α-Amyrin, β-Amyrin, 6-Methoxy-7,8-methylenedioxycoumarin, β-Sitosterol, 5,6,7-Trimethoxycoumarin

## INTRODUCTION

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

The compounds such as terpenoids, flavonoids, coumarins, acetylenes, caffeoylquinic acids and sterols were isolated and the various biological activities were investigated from *Artemisia* species (Tan *et al.*, 1998). The recent strudy on the compounds of *A. apiacea* shows the presence of campesterol, stigmasterol, β-sitosterol, 7-methoxycoumarin, 7,8-dimethoxycoumarin and 7,8-methylenedioxycoumarin in the flower heads (Shimomura *et al.*, 1979); daphnetin, 7-hydroxy-8-methoxycoumarin and 7-isopentenyloxy-8-methoxycoumarin in the flower heads (Shimomura *et al.*, 1980a); scopoletin, protocatechualdehyde and ethyl and methyl caffeates in the stems and leaves (Shimomura *et al.*, 1980b) and volatile constituents like α-pinene and artemisia ketone in the roots (Yano, 1970; Kim and Jang, 1994).

In the previous paper, we reported the isolation of artemicapin C, daucosterol, apigenin and cacticin (Lee *et al.*, 2002) and a new coumarin, arteminin (Kim *et al.*, 2002) from *A. apiacea*. Also we reported the hair-growth activity (Kim *et al.*, 1999) and the anti-oxidant activities of the extracts from the herbs (Kim *et al.*, 2003) of *A. apiacea*.

This paper describes the isolation and structural determination of constituents from the non-polar fraction of *A. apiacea*.

Correspondence to: Bak-Kwang Kim, College of Pharmacy, Seoul National University, Seoul 151-742, Korea
Tel: 82-2-880-7841, Fax: 82-2-878-1652
E-mail: kimbk2@snu.ac.kr

## MATERIALS AND METHODS

### 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. $^1$H- and $^{13}$C-NMR spectra were recorded with BRUKER AVANCE 400 NMR spectrometer in $CDCl_3$ using TMS as the internal standard. MS spectra were measured with JEOL JMS-AX505WA mass spectrometer. Other reagents were commercial grade without purification.

### Plant materials

The herbs of *Artemisia apiacea* Hance was purchased from the Kyungdong market, Korea in January 1999, and verified by Prof. Emeritus D. S. Han, Seoul National University, Korea. A voucher specimen of this plant has been deposited at the Herbarium of College of Pharmacy, Seoul National University, Korea.

### Extraction and isolation

The air-dried powdered herbs (5 kg) of *A. apiacea* 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. Each fraction was evaporated *in vacuo* to yield the residues of *n*-hexane fraction (40 g), $CH_2Cl_2$ fraction (38 g), EtOAc fraction (56 g) and *n*-BuOH fraction (30 g).

The portion of *n*-hexane fraction (30 g) was chromatographed on silica gel column eluting with a gradient of *n*-hexane-EtOAc to afford compounds **1** (9 mg), **2** (6 mg), **3** (7 mg), **4** (12 mg) and **5** (15 mg).

Compound **1**; EI-MS *m/z* (rel. int., %): 426 $[M]^+$ (7.0), 411 (5.9), 408 (8.2), 218 (100), 207 (3.9), 205 (12.0), 204 (11.6), 203 (18.6), 191 (9.6), 189 (12.5), 175 (6.6), 161 (8.1), 147 (9.2), 135 (11.0), 133 (9.8), 123 (15.1), 121 (13.5), 109 (16.9), 107 (12.4); $^1$H-NMR (400 MHz, $CDCl_3$) $\delta_H$ (ppm): 5.13 (1H, t, *J* = 4.0 Hz, H-12), 3.32 (1H, m, H-3α), 1.07, 1.00, 0.99, 0.95 (each 3H, s, H-27,26,23,25), 0.91 (3H, br s, H-30), 0.79 (6H, s, H-28,24), 0.78 (3H, d, *J* = 6.0 Hz, H-29); $^{13}$C-NMR (100 MHz, $CDCl_3$) $\delta_C$ (ppm): 139.6 (C-13), 124.4 (C-12), 79.1 (C-3), 59.1 (C-18), 55.2 (C-5), 47.7 (C-9), 42.1 (C-14), 41.6 (C-22), 40.0 (C-8), 39.8 (C-19), 39.6 (C-20), 38.8 (C-1,4), 36.9 (C-10), 33.8 (C-17), 32.8 (C-7), 31.1 (C-21), 28.8 (C-28), 28.1 (C-16,23), 27.2 (C-2), 26.6 (C-15), 23.4 (C-11), 23.3 (C-27), 21.4 (C-30), 18.4 (C-6), 17.5 (C-29), 16.8 (C-26), 15.6 (C-24,25).

Compound **2**; EI-MS *m/z* (rel. int., %): 426 $[M]^+$ (27.0), 411 (7.5), 408 (6.1), 393 (7.6), 218 (100), 207 (10.0), 203 (32.5), 191 (18.2), 189 (22.3), 175 (10.1), 161 (16.5), 141 (5.9), 135 (25.6), 133 (19.2), 123 (30.6), 121 (19.9), 119 (19.6), 109 (35.2), 107 (23.4); $^1$H-NMR (400 MHz, $CDCl_3$) $\delta_H$ (ppm): 5.15 (1H, t, *J* = 4.0 Hz, H-12), 3.22 (1H, m, H-3α), 1.14, 1.00, 0.97, 0.94 (each 3H, s, H-27,26,23,25), 0.88 (6H, s, H-30,29), 0.83, 0.79 (each 3H, s, H-28,24); $^{13}$C-NMR (100 MHz, $CDCl_3$) $\delta_C$ (ppm): 145.2 (C-13), 121.7 (C-12), 79.1 (C-3), 55.2 (C-5), 47.7 (C-9), 47.2 (C-18), 46.8 (C-19), 41.7 (C-14), 38.8 (C-4,8), 38.6 (C-1), 37.2 (C-22), 37.0 (C-10), 34.7 (C-21), 33.4 (C-29), 32.8 (C-7), 32.5 (C-17), 31.1 (C-20), 28.4 (C-28), 28.1 (C-23), 27.2 (C-2), 27.0 (C-16), 26.2 (C-15), 26.0 (C-27), 23.7 (C-30), 23.5 (C-11), 18.4 (C-6), 16.9 (C-26), 15.7 (C-25), 15.5 (C-24).

Compound **3**; EIMS *m/z* (rel. int. %): 414 $[M]^+$ (100), 396 (43.1), 329 (36.7), 303 (35.0), 273 (31.8), 255 (60.2), 213 (37.4), 199 (15.2), 159 (37.7), 145 (37.9); $^1$H-NMR (400 MHz, $CDCl_3$) $\delta_H$ (ppm): 5.35 (1 H, br d, *J* = 5.1 Hz, H-6), 3.53 (2 H, m, H-3), 1.03 (3 H, s, H-19), 0.94 (3 H, d, *J* = 6.6 Hz, H-21), 0.85 (3 H, t, *J* = 7.6 Hz, H-29), 0.83 (3 H, d, *J* = 7.3 Hz, H-26), 0.79 (3 H, d, *J* = 6.8 Hz, H-27) 0.68 (3 H, s, H-18); $^{13}$C-NMR (100 MHz, $CDCl_3$) $\delta_C$ (ppm): 140.7 (C-5), 121.7 (C-6), 71.8 (C-3), 56.8 (C-14), 56.0 (C-17), 50.1 (C-9), 45.8 (C-24), 42.3 (C-13), 40.5 (C-12), 39.7 (C-4), 37.2 (C-1), 36.5 (C-10), 36.1 (C-20), 33.9 (C-22), 31.8 (C-7), 31.6 (C-8), 29.7 (C-2), 29.1 (C-25), 28.3 (C-16), 26.0 (C-23), 24.3 (C-15), 23.1 (C-28), 21.1 (C-11), 19.8 (C-26), 19.4 (C-27), 19.0 (C-19), 18.8 (C-21), 12.2 (C-29), 12.0 (C-18).

Compound **4**; EI-MS *m/z* (rel. int. %): 236 $[M]^+$ (100), 221 (95.8), 193 (30.0), 178 (8.9), 161 (4.9), 150 (7.8); $^1$H-NMR (400 MHz, $CDCl_3$) $\delta_H$ (ppm): 7.95 (1H, d, *J* = 9.6 Hz, H-4), 6.63 (1H, s, H-8), 6.25 (1H, d, *J* = 9.6 Hz, H-3), 4.05 (3H, s, 5-$OCH_3$), 3.94 (3H, s, 6-$OCH_3$), 3.88 (3H, s, 7-$OCH_3$); $^{13}$C-NMR (100 MHz, $CDCl_3$) $\delta_c$ (ppm): 161.3 (C-2), 157.2 (C-7), 151.5 (C-5), 149.3 (C-9), 138.9 (C-4), 138.1 (C-6), 112.5 (C-3), 107.2 (C-10), 95.5 (C-8), 61.8 (5-$OCH_3$), 61.2 (7-$OCH_3$), 56.3 (6-$OCH_3$).

Compound **5**; EIMS *m/z* (rel. int. %): 220 $[M]^+$ (100), 192 (26.8), 177 (11.7), 163 (3.0), 149 (7.7), 147 (15.2), 121 (11.4), 107 (6.1), 79 (15.3); $^1$H-NMR (400 MHz, $CDCl_3$) $\delta_H$ (ppm): 7.57 (1H, d, *J* = 9.6 Hz, H-4), 6.58 (1H, s, H-5), 6.27 (1H, d, *J* = 9.6 Hz, H-3), 6.17 (2H, s, -$OCH_2O$-), 3.93 (3H, s, 6-$OCH_3$); $^{13}$C-NMR (100 MHz, $CDCl_3$) $\delta_c$ (ppm): 159.7 (C-2), 143.6 (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 (-$OCH_2O$-), 56.8 (6-$OCH_3$).

## RESULTS AND DISCUSSION

The chromatographic separation of the non-polar fraction from *A. apiacea* led to the isolation of α-amyrin (**1**), β-amyrin (**2**), β-sitosterol (**3**), 5,6,7-trimethoxycoumarin (**4**) and 6-methoxy-7,8-methylenedioxycoumarin (**5**).

Compounds **1** and **2** were obtained as white crystals. They responded positively to the Libermann-Burchard test. Mass spectral analysis showed the same molecular ion peak at *m/z* 426. In the $^1$H-NMR spectrum of compound **1**, the six angular methyl singlet signals at δ 1.07, 1.00, 0.99, 0.95 (each 3H, s, H-27,26,23,25) and 0.79 (6H, s, H-28,24), and the two secondary methyl doublet signals at δ 0.91 (3H, br s, H-30) and 0.78 (3H, d, *J* = 6.0 Hz, H-29) were observed, respectively. In contrast, the eight angular methyl singlet signals at δ 1.14, 1.00, 0.97, 0.94 (each 3H, s, H-27,26,23,25), 0.88 (6H, s, H-30,29), 0.83, 0.79 (each 3H, s, H-28,24) were observed from the $^1$H-NMR spectrum of compound **2**. Another independent supporting data was available from $^{13}$C-NMR spectrum. The chemical shifts at δ 139.6 and 124.4 showed the C-13 and C-12 of (C) ring of compound **1**, respectively. Also the chemical shifts at δ 145.2 and 121.7 showed that of compound **2**. Accordingly, the structures of compounds **1** and **2** were elucidated as α-amyrin and β-amyrin, respectively. A compilation of the $^{13}$C-NMR data of pentacyclic triterpenoids such as amyrins is provided (Kang, 1987; Mahato and Kundu, 1994).

Compound **3** was obtained as white crystals. In the EIMS, the molecular ion peak showed at *m/z* 414. In the

$^1$H-NMR spectrum, the angular methyl singlet signals of 18-Me and 19-Me at δ 0.68 and δ 1.03, and the doublet of 21-Me, 26-Me and 27-Me at δ 0.94, δ 0.83 and δ 0.79 were observed, respectively. The broad doublet at δ 5.35 showed H-6 of olefinic proton. In the $^{13}$C-NMR spectrum, the chemical shifts at δ 140.7 and 121.7 showed the C-5 and C-6 of (B) ring. Accordingly, the structure of compound **3** was elucidated as β-sitosterol. Chang *et al.* (1981) reported the $^{13}$C-NMR assignment of β-sitosterol and β-sitosteryl-3-*O*-β-D-glucopyranoside. Shimomura *et al.* (1979) already reported the presence of β-sitosterol from the flower heads of *A. apiacea*.

Compounds **4** and **5** were obtained as yellow-white crystals. Compounds **4** and **5** were visualized by the typical fluorescence on TLC plate. Initial identification of compounds **4** and **5** was accomplished by analysis of their $^1$H-NMR data. In the $^1$H-NMR spectrum of compound **4**, a pair of doublets at δ 6.25 and 7.95 (each 1H, d, $J$ = 9.6 Hz) showed the signals of H-3 and H-4 of a α-pyrone ring system. The aromatic region in the spectra additionally displayed a one-proton singlet at δ 6.63 consistent with a tri-substitution pattern on the aromatic ring. Independent support for the degree of aromatic functionalization was evident from the presence of three aromatic methoxy resonances at δ 3.88, 3.94 and 4.05 in the $^1$H-NMR spectrum. Mass spectral analysis showed the molecular ion at *m/z* 236. Detection of a long-range coupling between H-4 signal and C-8 signal confirmed that C-8 was unsubstituted and, hence, the 5,6,7-oxygenation pattern. Independent supporting evidence for the arrangement of the methoxy groups on the coumarin skeleton was available from $^{13}$C-NMR spectral data. The chemical shifts at δ 56.3, 61.2 and 61.8 of the aromatic methoxy groups clearly established the presence of tri-substituents. Accordingly, the structure of compound **4** was elucidated as 5,6,7-trimethoxycoumarin. Kayser and Kolodziej reported the isolation of highly oxygenated coumarins such as 5,6,7-trimethoxycoumarin from *Pelargonium sidoides* (1995).

In the $^1$H-NMR spectrum of compound **5**, a pair of doublets at δ 6.27 and 7.57 (each 1H, d, $J$ = 9.6 Hz) showed the signals of H-3 and H-4 of a α-pyrone ring system like that of compound **4**. The H-5 of aromatic region in the spectrum additionally displayed the one-proton singlet at δ 6.58. Independent support for the degree of aromatic functionalization was evident from the presence of an aromatic methoxy resonance at δ 3.93 and a methylenedioxy resonance at δ 6.17 in the $^1$H-NMR spectrum. Mass spectral analysis showed the molecular ion at *m/z* 220. The position of the methoxy group was confirmed by detection of a long-range coupling between C-6 and the proton of the methoxy group. Independent supporting evidence for the arrangement of the methoxy group and the methylenedioxy group on the coumarin skeleton was available from $^{13}$C-NMR spectral data. The chemical shifts at δ 56.8 and δ 103.5 of the aromatic ring clearly established the presence of the aromatic methoxy group and the methylenedioxy group, respectively. Accordingly, the structure of compound **5** was elucidated as 6-methoxy-7,8-methylenedioxycoumarin. Herz *et al.* reported the isolation of 6-methoxy-7,8-methylenedioxycoumarin from *Artemisia arctica* (1970).

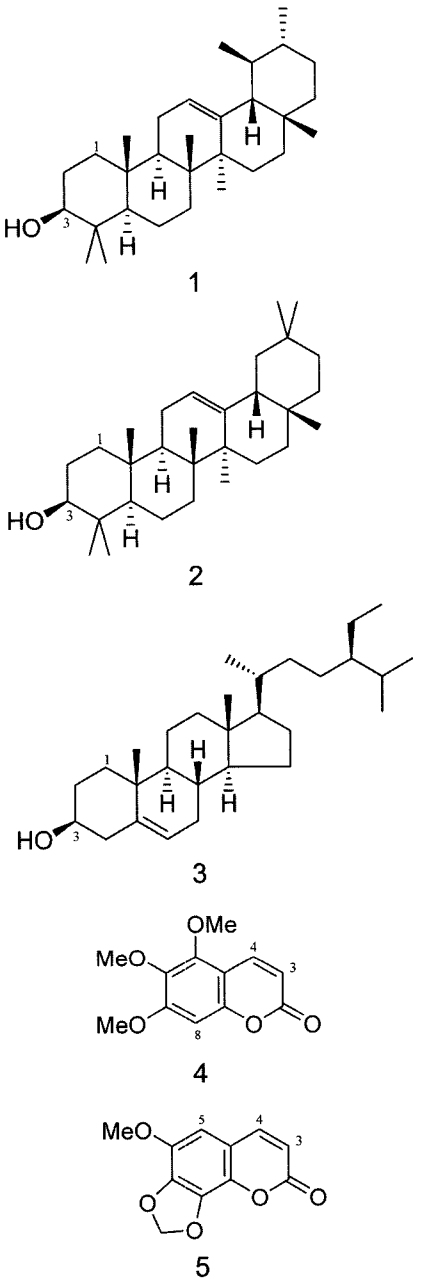


**Fig. 1.** Structures of compounds **1-5**

Their structures were shown in Fig. 1. Among the isolated compounds, this is the first report of the isolation of α-amyrin (**1**), β-amyrin (**2**), 5,6,7-trimethoxycoumarin (**4**) and 6-methoxy-7,8-methylenedioxycoumarin (**5**) from *A. apiaceae*.

## ACKNOWLEDGEMENT

This work was partly supported by BK21 and Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Korea.

## REFERENCES

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).

Herz, W., Bhat, S. V., and Santhanam, P. S., Coumarins of *Artemisia dracunculoides* and 3',6-dimethoxy-4',5,7-trihydroxyflavone in *A. arctica*. *Phytochemistry,* 9, 891-894 (1970).

Kang, S. S., $^{13}$C-NMR spectroscopy of amyrins. *Kor. J. Pharmacogn.,* 18, 151-167 (1987).

Kayser, O. and Kolodziej, H., Highly oxygenated coumarins from *Pelargonium sidoides*. *Phytochemistry,* 39, 1181-1185 (1995).

Kim, K. S., Lee, S., Lee, Y. S., Jung, S. H., Park, Y., Shin, K. H., and Kim, B.-K., Anti-oxidant activities from the herbs of *Artemisia apiacea*. *J. Ethnopharmacol.,* 85, 69-72 (2003).

Kim, K. S., Lee, S., Shin, J. S., Shim, S. H., and Kim, B.-K., Arteminin, a new coumarin from *Artemisia apiacea*. *Fitoterapia,* 73, 266-268 (2002).

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, S., Kim, K. S., Jang, J. M., Park, Y., Kim, Y. B., and Kim, B.-K., Phytochemical constituents from the herba of *Artemisia apiacea*. *Arch. Pharm. Res.,* 25, 285-288 (2002).

Mahato, S. B. and Kundu, A. P., $^{13}$C-NMR spectra of pentacyclic triterpenoids A compilation and some salient features. *Phytochemistry,* 37, 1517-1575 (1994).

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).

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

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).