**Natural Product Sciences**
11(1) : 10-12 (2005)

# Flavonol Galactosides from *Artemisia apiacea*

**Kyoung Soon Kim[1], Sanghyun Lee[1]*, Kyoung-Hwan Kang[2], and Bak-Kwang Kim[1]**

[1]*College of Pharmacy, Seoul National University, Seoul 151-742, Korea*
[2]*Lotte Pharm. Co. Ltd., Hwasung 445-746, Korea*

**Abstract** – Flavonol galactosides were isolated from the EtOAc fraction of *Artemisia apiacea* by repeated column chromatography. Their structures were elucidated as isorhamnetin-3-*O*-β-D-galactoside (**1**) and quercetin-3-*O*-β-D-galactoside (**2**) by chemical and spectroscopic analysis. This is the first report on the isolation of compound **2** from this plant.
**Keywords** – *Artemisia apiacea*, Compositae, flavonol galactoside, isorhamnetin-3-*O*-β-D-galactoside, quercetin-3-*O*-β-D-galactoside

## Introduction

*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 such as terpenoids, flavonoids, coumarins, acetylenes, caffeoylquinic acids and sterols were isolated and various biological activities were investigated from *Artemisia* species (Tan *et al.*, 1998). 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 (Shimomura *et al.*, 1979), daphnetin, 7-hydroxy-8-methoxycoumarin and 7-isopentenyloxy-8-methoxycoumarin from the flower heads (Shimomura *et al.*, 1980a), scopoletin, protocatechualdehyde and ethyl and methyl caffeates from the stems and leaves (Shimomura *et al.*, 1980b) and volatile constituents like α-pinene and artemisia ketone from the roots (Yano, 1970; Kim and Jang, 1994).

In previous papers, we reported the isolation of artemicapin C, daucosterol, apigenin and cacticin (Lee *et al.*, 2002), a new coumarin arteminin (Kim *et al.*, 2002) and α-amyrin, β-amyrin, β-sitosterol, 5,6,7-trimethoxycoumarin and 6-methoxy-7,8-methylenedioxycoumarin (Lee *et al.*, 2003a) from *A. apiacea*. Also we reported the hair-growth activity (Kim *et al.*, 1999) and the antioxidant activities (Kim *et al.*, 2003) of *A. apiacea*. In this paper, we describe the isolation and structural determination of flavonol galactosides from the EtOAc fraction of *A. apiacea*.

## Experimental

**Instruments and reagents** – Silica gel 60 (MERCK Co., 0.063-0.200 mm) was used for repeated 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 a JASCO FT/IR-300E instrument on KBr disc. $^1$H- and $^{13}$C-NMR spectra were recorded with a BRUKER AVANCE 400 NMR spectrometer in DMSO-$d_6$ using TMS as an internal standard. Chemical shifts were reported in parts per million (δ), and coupling constants (*J*) were expressed in hertz (Hz). MS spectra were measured with a 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 (Voucher No. Kim 99015) 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

*Author for correspondence
Fax: +82-2-880-7841; E-mail: jnp@korea.com

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 (40 g), $CH_2Cl_2$ (38 g), EtOAc (56 g) and *n*-BuOH fractions (30 g).

A portion of the EtOAc fraction (20 g) was chromatographed on silica gel column eluting with a gradient of $CHCl_3$-MeOH to afford compounds **1** (64 mg, 90:10) and **2** (42 mg, 90:10).

Compound **1**; EI-MS (70 eV): *m/z* 316 [isorhamnetin]$^+$; FAB-MS: *m/z* 479 $[M+H]^+$; IR $\nu_{max}$ (KBr) $cm^{-1}$: 3421 (OH), 1625 (C=O), 1092 (C-O); $^1$H-NMR (400 MHz, DMSO) and $^{13}$C-NMR (100 MHz, DMSO): see Tables 1 and 2, respectively.

Compound **2**; EI-MS (70 eV): *m/z* 302 [quercetin]$^+$; FAB-MS: *m/z* 465 $[M+H]^+$; IR $\nu_{max}$ (KBr) $cm^{-1}$: 3380 (OH), 1619 (C=O), 1020 (C-O); $^1$H-NMR (400 MHz, DMSO) and $^{13}$C-NMR (100 MHz, DMSO): see Tables 1 and 2, respectively.

**Acid hydrolysis of compounds 1 and 2** – Compounds **1** and **2** (each 10 mg) was refluxed with 5% $H_2SO_4$ in MeOH (3 ml) for 4 hr. Workup in the usual way, followed by crystallization afforded galactose (co-TLC, *n*-BuOH: HOAc:$H_2O$ = 4:1:5) and aglycones identified as isorhamnetin (**1a**) and quercetin (**2a**), respectively.

Compound **1a**; EI-MS (rel. int. %): *m/z* 316 $[M]^+$; $^1$H-NMR (400 MHz, DMSO) and $^{13}$C-NMR (100 MHz, DMSO): see Tables 1 and 2, respectively.

Compound **2a**; EI-MS (rel. int. %): *m/z* 302 $[M]^+$; $^1$H-NMR (400 MHz, DMSO) and $^{13}$C-NMR (100 MHz, DMSO): see Tables 1 and 2, respectively.

## Results and Discussion

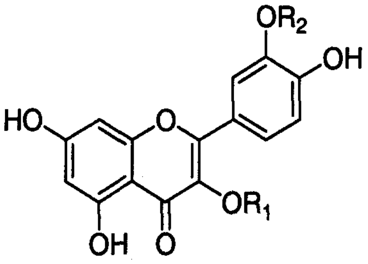
A chromatographic separation of the EtOAc fraction from *A. apiacea* led to the isolation of compounds **1** and **2**. Compounds **1** and **2** were obtained as yellow crystals from MeOH. They responded positively to the Shinoda and the Molisch test. The spectra of compounds **1** and **2** were similar to each other. In the EIMS, the aglycone peaks of compounds **1** and **2** showed at *m/z* 316 and 302, respectively. The characteristic fragment ion peaks at *m/z* 153 and 121 in the EIMS showed the *retro* Diels Alder fragmentation of flavonoids (Markham, 1982). The aglycones of compounds **1** and **2** were identified as isorhamnetin (**1a**) and quercetin (**2a**), respectively, by chemical reaction (acid hydrolysis). The FABMS of compounds **1** and **2** showed $[M+H]^+$ peak at *m/z* 479 and 465 corresponding to the molecular formula $C_{22}H_{22}O_{12}$

**Table 2.** $^{13}$C-NMR data of compounds **1** and **2**

| No. | 1 | 1a | 2 | 2a |
|---|---|---|---|---|
| 2 | 156.4 | 156.3 | 156.3 | 156.6 |
| 3 | 133.1 | 133.5 | 133.5 | 133.9 |
| 4 | 177.5 | 176.4 | 177.4 | 177.9 |
| 5 | 161.2 | 161.5 | 161.2 | 161.6 |
| 6 | 98.7 | 98.6 | 98.7 | 99.1 |
| 7 | 164.2 | 163.3 | 164.2 | 164.5 |
| 8 | 93.7 | 93.6 | 93.5 | 93.9 |
| 9 | 156.2 | 156.1 | 156.2 | 156.6 |
| 10 | 104.0 | 103.9 | 103.9 | 104.3 |
| 1' | 121.1 | 121.2 | 121.1 | 121.5 |
| 2' | 113.5 | 112.4 | 115.9 | 115.6 |
| 3' | 146.9 | 147.0 | 144.8 | 145.2 |
| 4' | 149.4 | 149.3 | 148.5 | 148.9 |
| 5' | 115.2 | 115.3 | 115.2 | 116.3 |
| 6' | 122.0 | 122.0 | 121.9 | 122.4 |
| 1'' | 101.6 | - | 101.8 | - |
| 2'' | 71.2 | - | 71.2 | - |
| 3'' | 73.1 | - | 73.2 | - |
| 4'' | 67.9 | - | 67.9 | - |
| 5'' | 75.8 | - | 75.9 | - |
| 6'' | 60.1 | - | 60.3 | - |
| 3'-OMe | 56.0 | 55.6 | - | - |

**Table 1.** $^1$H-NMR data (*J* Hz) of compounds **1** and **2**

| No. | 1 | 1a | 2 | 2a |
|---|---|---|---|---|
| 6 | 6.20 (d 1.9) | 6.19 (d 2.0) | 6.16 (d 1.9) | 6.21 (d 1.9) |
| 8 | 6.44 (d 1.9) | 6.45 (d 2.0) | 6.40 (d 1.9) | 6.41 (d 1.9) |
| 2' | 8.02 (d 2.0) | 7.93 (d 1.9) | 7.57 (d 1.9) | 7.53 (d 2.0) |
| 5' | 6.90 (d 8.5) | 6.90 (d 8.3) | 6.81 (d 8.5) | 6.82 (d 8.5) |
| 6' | 7.67 (dd 2.0, 8.5) | 7.67 (dd 1.9, 8.3) | 7.67 (dd 1.9, 8.5) | 7.67 (dd 2.0, 8.5) |
| 1'' | 5.52 (d 7.7) | - | 5.37 (d 7.7) | - |
| 5-OH | 12.62 (s) | 12.62 (s) | 12.63 (s) | 12.64 (s) |
| 3'-OMe | 3.84 (s) | 3.84 (s) | - | - |



**1** $R_1$ = Gal, $R_2$ = Me

**1a** $R_1$ = H, $R_2$ = Me

**2** $R_1$ = Gal, $R_2$ = H

**2a** $R_1$ = H, $R_2$ = H

**Fig. 1.** Structures of compounds **1** and **2.**

and $C_{21}H_{20}O_{12}$, respectively. In the $^1$HNMR spectra of compounds **1** and **2**, the typical flavonoid signals were observed. Two *meta*-coupled signals H-6 and -8 of (A) ring and three ABX type signals H-2', -5' and -6' due to (B) ring were observed (see Table 1). The anomeric protons of compounds **1** and **2** were observed at δ 5.52 (d, 7.7 Hz) and 5.37 (d, 7.7 Hz), respectively. The singlets of aromatic 5-OH of compounds **1** and **2** at δ 12.62 and 12.63 were observed, respectively. The position of galactose of both compounds and a methoxyl group of compound **1** was conducted by HMBC analysis. The $^{13}$CNMR spectra of compounds **1** and **2** showed C=O at δ 177.5 and 177.4, respectively, and carbons of galactose (see Table 2). The carbon signal at δ 101.6 and 101.8 showed C-1" of galactose of compounds **1** and **2**, respectively. The IR spectra of compounds **1** and **2** showed absorption bands for hydroxyl at 3421 and 3380 $cm^{-1}$, respectively.

Accordingly, the structures of compounds **1** and **2** were elucidated as isorhamnetin-3-*O*-β–D-galactoside and quercetin-3-*O*-β–D-galactoside, respectively, by comparing their spectral data in the literature (Lee *et al.*, 2002; Lee *et al.*, 2003b). This is the first report on the isolation of compound **2** from this plant.

## Acknowledgment

This work was supported in part by a grant from Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Korea.

## References

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).

Lee, S., Kim, K.S., Shim, S.H., Park, Y.M., and Kim, B.K., Constituents from the non-polar fraction of *Artemisia apiacea. Arch. Pharm. Res.* **26**, 902-905 (2003a).

Lee, S., Shin, D.S., Oh, K.B., and Shin, K.H., Antibacterial compounds from the leaves of *Acanthopanax senticosus. Arch. Pharm. Res.* **26**, 40-42 (2003b).

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

Shimomura, H., Sashida, Y., and Ohshima, Y., Coumarins from *Artemisia apiacea. Phytochem.* **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).

(Accepted March 5, 2005)