J. Korean Soc. Appl. Biol. Chem. **51**(4), 305-308 (2008)

Article

Phytochemical Constituents of the Roots of *Erigeron annuus*

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Received August 13, 2008; Accepted October 8, 2008

Seven compounds (1-7) were isolated from *n*-hexane and EtOAc-soluble fractions of the roots of *Erigeron annuus* by repeated silica gel column chromatography. They were identified as simiarenol (1), β-sitosterol (2), daidzein (3), apigenin (4), apigenin 7-*O*-β-D-glucuronide (5), 3-hydroxy-pyran-4-one (6), and β-sitosterol glucoside (7) on the basis of physical and spectroscopic data. Compounds 1 and 3 were isolated for the first time from the *Erigeron* species.

Key words: *Compositae,* Erigeron annuus*, phytochemical constituents*

The genus *Erigeron* is a member of the Compositae (Asteraceae) family and contains more than 400 species that occur widely in temperate North America, South America, East Asia, and Europe [Bennington *et al*., 1998]. Some of these have a long history of applications in Korean and Chinese folk medicines, especially *Erigeron annuus* (L.) Per., traditionally used for the treatment of indigestion, enteritis, epidemic, hepatitis, and hematuria [Jiangsu College of New Medicine, 1977]. Previous phytochemical investigations on this plant resulted in the isolation of γ-pyranone derivatives [Hashidoko, 1995; Oh *et al*., 2002; Li *et al*., 2005], cyclopentenone derivatives [Iijima *et al*., 2003a], sesquiterpenoids [Iijima *et al*., 2003b; Li *et al*., 2005], flavonoids [Hashidoko, 1995], triterpenoids [Hashidoko, 1995], and phenolic derivatives [Oh *et al*., 2002; Li *et al*., 2006]. Recently, flavonoids [Yoo *et al*., 2008], γ-pyranone derivatives [Yoo *et al*., 2008], and phenolic compounds [Jang *et al*., 2008] have been isolated from the flowers of this plant by our group. However, a systematic phytochemical study on the roots has not been carried out yet. We now report on the isolation and identification of seven compounds (**1-7**) from the roots of *E. annuus*.

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Abbreviations: CC, column chromatography; TLC, thin layer chromatography

doi:10.3839/jksabc.2008.053

Materials and Methods

Plant materials. The roots of *E. annuus* were collected from Daejeon, Korea, in June 2006 and were identified by a plant taxonomist, Prof. Joo-Hwan Kim, Division of Life Science, Daejeon University, Daejeon, Korea. A voucher specimen (KIOM-ERAN2) has been deposited at the Herbarium of the Department of Herbal Pharmaceutical Development, Korea Institute of Oriental Medicine, Korea.

Instruments. Melting points were measured on an IA9100 melting point apparatus (Barnstead International, Dubuque, IA) and were quoted uncorrected. Optical rotations were obtained using a P-2000 digital polarimeter (JASCO, Tokyo, Japan). NMR experiments were conducted on a DRX-300 FT-NMR (Bruker, Karlsruhe, Germany), and the chemical shifts were referenced to the residual solvent signals. TLC analyses were performed on Kieselgel 60 F_{254} (Merck, Darmstadt, Germany) plates (silica gel, 0.25 mm layer thickness); compounds were visualized by dipping plates into 10% (v/v) H_2SO_4 reagent (Aldrich, St. Louis, MO) and then heated at 110°C for 5-10 min. Silica gel (Merck 60A, 70-230 or 230-400 mesh ASTM), Sephadex LH-20 (Amersham Pharmacia Biotech, Uppsala, Sweden), and reversed-phase silica gel (YMC Co, Kyoto, Japan; ODS-A 12 nm S-150 μm) were used for column chromatography. All solvents used for the chromatographic separations were distilled before use.

Extraction and isolation. The dried and cut plant materials (1.25 kg) were extracted with MeOH (3×13 L) by maceration at room temperature for 3 days. The extracts were combined and concentrated *in vacuo* at

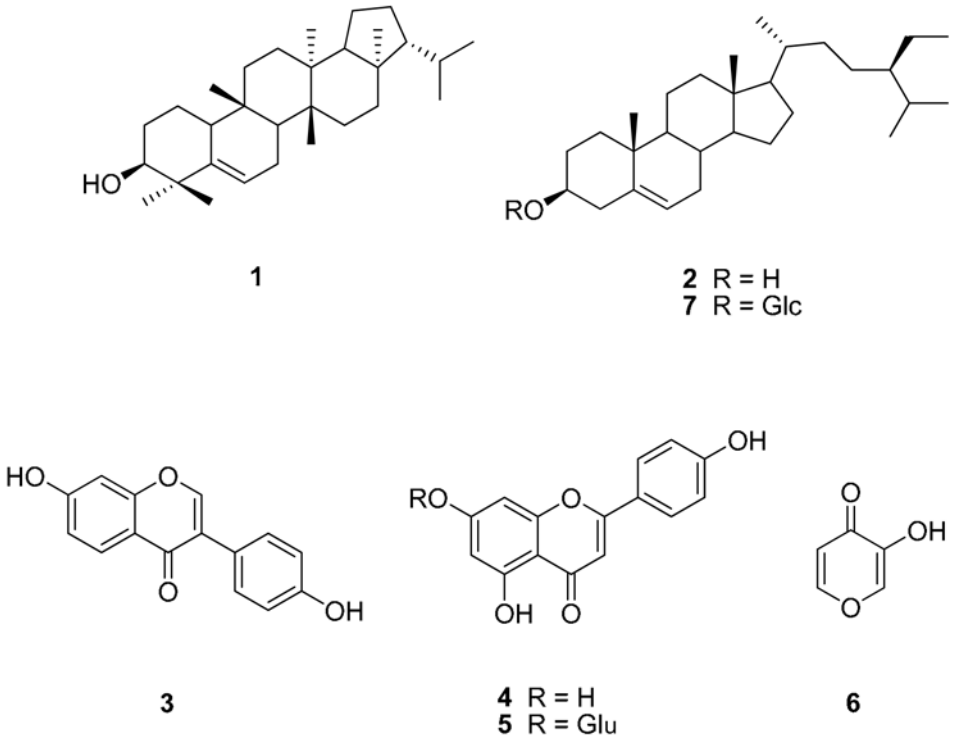

Fig. 1. Structures of compounds 1-7 from the roots of *Erigeron annuus*.

40°C. The concentrated extract (61 g) was suspended in H_2O (1 L) and partitioned successively with *n*-hexane (3 ×1 L) and EtOAc (3×1 L) to afford the *n*-hexane- (6.04 g), EtOAc- (3.32 g), and H_2O-soluble fractions (51.64 g). The *n*-hexane-soluble fraction was chromatographed through silica gel (7.0×40 cm, 70-230 mesh) using an *n*-hexane/EtOAc gradient from 20:1→1:1 (v/v) and finally with 100% MeOH to yield 17 fractions (H01-H17). Simiarenol (**1**, 19 mg) and β-sitosterol (**2**, 8 mg) were purified from fractions H05 (80 mg) and H10 (100 mg) by recrystallization, respectively. The EtOAc-soluble fraction was chromatographed through silica gel (5.0×50 cm, 70-230 mesh) using a $CHCl_3$/MeOH/H_2O gradient from 10:1:0.1→7:4:1 (v/v) and finally with 100% MeOH to yield 13 fractions (E01-E13). Fraction E03 (400 mg) was further fractionated using Sephadex LH-20 CC (5.0 ×44 cm, MeOH) to afford ten subfractions (E0301-E0310). Fraction 0307 (5.5 mg) was subjected to reversed phase CC (2.8×38 cm, 12 nm S-150 μm; MeOH/H_2O= 6:4) to give daidzein (**3**, 1.1 mg). Apigenin (**4**, 16 mg) was obtained from fraction E0309. β-Sitosterol glucoside (**7**, 20 mg) was purified from fraction E05 by recrystallization (MeOH). The remaining E05 fraction was further purified over a Sephadex LH-20 CC (4.0×48 cm) with MeOH, yielding 3-hydroxy-pyran-4-one (**6**, 1.4 mg). Fraction E12 (860 mg) was further fractionated using Sephadex LH-20 CC (5.0×72 cm, MeOH) to give apigenin 7-*O*-β-D-glucuronide (**5**, 9 mg).

Simiarenol (1): White powder; mp 210-212C; $[\alpha]_D^{25}$ +55.76 (*c* 0.5, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 5.62 (1H, brd, *J*=5.7 Hz, H-6), 3.47 (1H, brs, H-3α), 1.15, 1.05, 1.01, 0.93 and 0.90 (each 3H, s, H-24, 23, 26, 27, 25), 0.87 (3H, brd, *J*=5.1 Hz, H-29), 0.84 (3H, d, *J*= 6.6, H-30), 0.79 (3H, s, H-28); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 142.2 (C-5), 122.2 (C-6), 76.5 (C-3), 60.2 (C-21), 51.9 (C-18), 50.4 (C-10), 44.4 (C-8), 43.0 (C-17), 41.0 (C-14), 39.5 (C-4), 38.8 (C-13), 35.6 (C-16), 35.0 (C-9), 34.3 (C-11), 30.9 (C-22), 29.3 (C-15), 29.29 (C-23), 29.23 (C-12), 28.5 (C-20), 28.0 (C-2), 25.6 (C-24), 24.2 (C-7), 23.1 (C-30), 22.1 (C-29), 20.1 (C-19), 18.2 (C-1), 18.0 (C-25), 16.2 (C-28), 15.9 (C-26), 15.2 (C-27).

Daidzein (3): Yellowish powder; ^{1}H-NMR (300 MHz, CD_3OD) δ 8.10 (1H, s, H-2), 8.04 (1H, d, *J*=9.0 Hz, H-5), 7.36 (2H, d, *J*=8.0 Hz, H-2', 6'), 6.92 (1H, dd, *J*=8.0, 2.0 Hz, H-6), 6.84 (2H, d, *J*=8.0 Hz, H-3', 5'), 6.81 (1H, d, *J* =2.0 Hz, H-8).

Results and Discussion

Seven compounds (**1-7**) were isolated from *n*-hexane and EtOAc-soluble fractions of the roots of *E. annuus* by repeated CC. Structural identifications of these compounds were carried out by interpretation of the spectral data and comparison with the data described in the literature. Compounds **2** and **7** were identified as a common plant sterol, β-sitosterol [Yoo *et al.*, 2007] and β-sitosterol

glucoside [Yoo *et al*., 2007], respectively. Compounds **4-6** were identified as apigenin [Han *et al*., 2003], apigenin 7-*O*-β-D-glucuronide [Hase *et al*., 1995] and 3-hydroxy-pyran-4-one [Oh *et al*., 2002]; the compounds have also been reported as constituents of the flowers of this plant [Yoo *et al*., 2008].

Compound **1** was obtained as a white powder. The ^{1}H-NMR spectrum of **1** showed an olefinic proton at δ 5.62 (1H, brd, *J*=5.7 Hz, H-6), a carbinol proton at δ 3.47 (1H, brs, H-3α), two methyl doublets at δ 0.84 and 0.87, and six methyl singlets at δ 0.79-1.15. The ^{13}C-NMR spectrum of **1** exhibited the presence of 30 carbon signals as well as 2 olefinic carbon signals at δ 122.2 and 142.2, and 1 oxygenated carbon signal at δ 76.5. The structure of **1** was determined to be a migrated hopane-type triterpene, simiarenol, by comparison of the above data with those published in the literature [Kwon *et al*., 2001]. Although simiarenol (**1**) has been isolated from *Artemisia stolonifera* [Kwon *et al*., 2001], *A. argyi* [Lao *et al*., 1984], and *Caraipa densifolia* [Gunasekera *et al*., 2001], to the best of our knowledge, the present study is the first to report on the isolation of simiarenol from the *Erigeron* species.

The ^{1}H-NMR spectrum of **3** showed a resonance for an isoflavone skeleton that exhibited a diagnostic vinylic singlet at δ 8.10 (1H, s, H-2). Two *ortho*-coupled doublets centered at δ 7.36 (2H, d, *J*=8.0 Hz, H-2', H-6') and 6.84 (2H, d, *J*=8.0 Hz, H-3', H-5') were assigned to the protons of a *para*-di-substituted benzene ring (B ring). A set of ABX-type signals at δ 8.04 (1H, d, *J*=9.0 Hz, H-5), 6.92 (1H, dd, *J*=8.0, 2.0 Hz, H-6), and 6.81 (1H, d, *J*=2.0 Hz, H-8) were also observed in the ^{1}H-NMR spectrum of **3**. On the basis of these evidences and comparison with the published data, **3** was determined to be daidzein [Jung *et al*., 2004]. Although daidzein is a well known phytoestrogen and is very common in the plant kingdom, especially Leguminoceae, there are only few reports on the presence of daidzein in Compositae [Cho *et al*., 2008]. The present study is the first to report on the isolation of daidzein from the *Erigeron* species.

Acknowledgment. We thank Prof. J. H. Kim, Division of Life Science, Daejeon University, Daejeon, Korea, for identifying the plant material. NMR and MS experiments were performed by the Korea Basic Science Institute (KBSI). This research was supported by a grant [L07010] from Korea Institute of Oriental Medicine.

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