Phytochemical Constituents of *Artemisia stolonifera*

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Repeated column chromatographic separation of the CH₂Cl₂ extract of *Artemisia stolonifera* (Asteraceae) led to the isolation of a triterpene (I), a sesquiterpene (II), two aromatic compounds (III and IV) and a benzoquinone (V). Their structures were determined by spectroscopic means to be simiarenol (I), (1S,7S)-1β-hydroxygermacra-4(15),5,10(14)-triene (II), 3'-methoxy-4'-hydroxy-trans-cinnamaldehyde (III), vanillin (IV) and 2,6-dimethoxy-1,4-benzoquinone (V), respectively. Among these products, compound V showed significant cytotoxicity against five human tumor cell lines *in vitro*, A549 (non small cell lung adenocarcinoma), SK-OV-3 (ovarian), SK-MEL-2 (skin melanoma), XF498 (CNS) and HCT15 (colon) with ED₅₀ values ranging from 1.33~4.22 µg/ml.

**Key words:** *Artemisia stolonifera*, Asteraceae, Cytotoxicity, Simiarenol, (1S,7S)-1β-Hydroxygermacra-4(15),5,10(14)-triene, 3'-Methoxy-4'-hydroxy-trans-cinnamaldehyde, Vanillin, 2,6-Dimethoxy-1,4-benzoquinone

**INTRODUCTION**

*Artemisia stolonifera* (Asteraceae), a perennial herb which is common in Korea, has long been used as a folk medicine to treat eye disease, fever and retention of urine (Lee, 1989; Song, 1990). We previously reported the isolation of phytosterol, phenolic compounds and cytotoxic sesquiterpene peroxides from *A. stolonifera* (Hong et al., 1995; Lee et al., 1996; Kwon et al., 2000). In continuation of our research on this plant, we isolated five known compounds from this plant. The structural characterization of the five compounds is described and their cytotoxic activities are evaluated in this paper.

**MATERIALS AND METHODS**

**General experimental procedure**

Melting points were measured in uncorrected form on a Gallenkamp melting point apparatus. The EIMS spectrum was measured on a VG70-VSEQ (VG ANALITICAL, UK), the IR spectrum with a Shimadzu IR-435, and the **H**- and **C**-NMR spectra with a Bruker AMX-500. The LPLC column was a Lobar® A Lichroprep Si-60 (Merck) and the pump was a DURAMAT 80 (Germany). The HPLC was a JAI-LC 908 model with a UV and refractive dual detector system and a connected JAIGEL-1H (20 x 600 mm) and JAIGEL-2H (20 x 600 mm) column. TLC was performed on precoated Kiesel gel 60 F₂₅₄ plates (Merck). The silica gel for column chromatography was Kiesel gel 60 (70-230 mesh, 230-400 mesh, Merck).

**Plant materials**

The aerial parts of *Artemisia stolonifera* (Max.) Kom. (Asteraceae) were collected at Mt. Kwang-Duck in Kyung-Gi-do, South Korea in August 1994. The voucher specimen (SKK-94-001) has been deposited in the herbarium of the College of Pharmacy, SungKyunKwan University, Suwon, KyungGi-Do, Korea.

**Cytotoxicity testing**

Sulforhodamin B Bioassay (SRB) was used for cytotoxicity evaluation. The activity of each compound was tested at several concentration levels against five cultured human tumor cells *in vitro* (Skehan et al., 1990); A549 (non small cell lung adenocarcinoma), SK-OV-3 (ovarian), SK-MEL-2 (skin melanoma), XF498 (CNS) and HCT15 (colon).
Extraction, separation and purification of compounds

The dried and chopped aerial parts of the plant (2 kg) were extracted with CH₄Cl₂ three times at room temperature. The concentrated CH₄Cl₂ extract (21 g) was subjected to column chromatography over silica gel and eluted sequentially with hexane-EtOAc (10:1, 1 L; 8:1, 1 L; 6:1, 500 ml; 3:1, 700 ml; 1:1, 1.5 L) and then with hexane-EtOAc-MeOH (20:20:1, 1.5 L; 5:5:1, 1 L; 2:5:1, 500 ml) to give eight subfractions ($1-$8: void volume (400 ml), $1 (500 ml), $2 (80 ml), $3 (220 ml), $4 (850 ml), $5 (1350 ml), $6 (900 ml) and $8 (2600 ml)). The $4 fraction (1.4 g) was chromatographed with hexane-EtOAc (8:1) and purified with a Lobar Si-60 column (hexane-EtOAc=8:1) to generate compound III (20 mg). The $5 fraction (3.4 g) was re-chromatographed over silica gel (hexane-CHCl₃-acetone=7:3:1) and purified with recyclic JAI HPLC (GPC column, CHCl₃, flow rate 3.5 ml/min) to generate compound I (10 mg, tR=43 rain).

Simiarenol (I). white powder; [δ]D₂O +46.2° (0.2, CHCl₃); mp 213°C; IR νmax (Nujol) cm⁻¹: 3400, 1660, 1580, 1250; UV λmax (CHCl₃) nm : 333, 332 (sh); EIMS m/z (rel. int.): 178 (M⁺,100), 161 (35), 147 (60), 135 (68), 107 (44), 84 (35), 77 (34); ¹H-NMR (500 MHz, CDCl₃) : δ 0.79 (3H, s, OCH₃), 5.86 (2H, s, H-3, 5); ¹³C-NMR (125 MHz, CDCl₃) : δ 60.08 (C-21), 76.39 (C-3), 122.03 (C-1), 19.95 (C-12), 21.98 (C-29), 22.93 (C-30), 24.10 (C-7), 25.49 (C-24), 27.81 (C-2), 28.34 (C-20), 29.03 (C-12), 29.09 (C-23), 29.15 (C-2), 30.81 (C-30), 34.18 (C-11), 34.86 (C-9), 35.45 (C-16), 38.65 (C-13), 39.36 (C-4), 40.84 (C-14), 42.83 (C-17), 44.30 (C-8), 50.28 (C-10), 51.18 (C-18), 60.08 (C-21), 76.39 (C-3), 122.03 (C-6), 142.03 (C-5)

(V). yellow needles; mp 250°C; UV λmax (CHCl₃) nm: 379, 287; MS m/z (rel. int.): 168 (M⁺,100), 153 (8), 138 (45), 125 (20), 97 (20), 80 (62), 69 (20), 59 (26), 53 (41); ¹H-NMR (500 MHz, CDCl₃) : δ 3.95 (3H, s, OCH₃), 6.20 (1H, s, OH), 7.06 (1H, d, J=8.4Hz, H-5), 7.44 (2H, m, H-2, H-6), 9.85 (1H, s, aldehyde H); ¹³C-NMR (125 MHz, CDCl₃) : δ 56.17 (OCH₃), 109.53 (C-5), 114.98 (C-2), 124.07 (C-6), 126.51 (C-2), 126.73 (C-1), 147.00 (C-4), 149.88 (C-3), 153.01 (C-3), 193.56 (C-1)

RESULTS AND DISCUSSION

Compound I was obtained as a white amorphous powder and its molecular formula was deduced to be C₁₉H₁₇O from EIMS (M⁺ m/z 426) and ¹³C-NMR data. The IR spectrum exhibited a hydroxy (3510 cm⁻¹) absorption band. The ¹H-NMR spectrum showed an olefinic proton at δ 5.62 (1H, brd, J=5.9 Hz, H-6), a carbonyl proton at δ 3.48 (1H, brs, H-3α), two methyl doublets at δ 0.83 and 0.89, and six methyl singlets at δ 0.79-1.15. The ¹³C-NMR spectrum exhibited the presence of 30 carbon atoms.
signals and also showed two olefinic carbon signals at δ 122.03 and 142.03, and an oxygenated carbon signal at δ 76.39. The above data were consistent with that for 3-hydroxy-Δ5-adienene type triterpene. The structure of I was determined to be simiarenoI on the basis of the above evidences, together with a comparison of the above data with those published in the literature (Tanaka et al., 1988).

Compound II was obtained as a colorless oil and showed a molecular ion peak at m/z 220 (C15H24O) and a base peak at m/z 109 in EIMS. The IR spectrum showed the presence of an OH group at 3300 cm⁻¹. The 1H-NMR spectrum showed two secondary methyl groups (at δ 0.84 and 0.92), an oxygenated methine proton (at δ 3.79), two olefinic protons (at δ 5.45 and 6.01), and four exomethylene protons (at δ 4.86, 4.94, 5.02 and 5.29). The 13C-NMR spectrum exhibited the presence of 15 carbon signals, consisting of six olefinic carbon signals at δ 110.54, 112.88, 129.69, 137.94, 146.79 and 153.59, one oxygenated carbon signal at δ 76.02, and eight aliphatic signals at δ 20.49, 20.74, 29.99, 34.54, 36.22, 36.23, 31.80 and 52.53. These spectral data suggested that II was a sesquiterpene with a secondary alcohol, two exomethylene and a trans double bond. Based on the reported chemical structures of the sesquiterpene (Bohlmann et al., 1982) and on NMR spectral data, II was determined to be (1S,7S)-1β-hydroxygermacra-4(15),5,10(14)-triene. The NMR spectral and physical data of compound II were in good agreement with those reported in the literatures (Bohlmann et al., 1982; Nagashima et al., 1990).

Compound III was obtained as a yellow powder. ElMS and DEPT data established a molecular formula of C20H10O3. The IR spectrum exhibited hydroxy (3400 cm⁻¹) and carbonyl groups (1660 cm⁻¹). The 1H- and 13C-NMR spectra indicated the presence of an aromatic ring, a trans double bond [at δ 6.61 (dd, J=15.9, 7.7 Hz) and δ 7.41 (d, J=15.9 Hz)], an aldehyde group at δ 9.65 (d, J=7.7 Hz) in the 1H-NMR spectrum and δ 193.59 in the 13C-NMR spectrum and a methoxy group at δ 3.97 in the 1H-NMR spectrum. By comparison of its spectral data with those of literature values (Herath et al., 1998), III was determined to be 3′-methoxy-4-hydroxy-trans-cinnamaldehyde.

Compound IV was obtained as a yellow powder and showed a molecular ion peak at m/z 152. In the 1H- and 13C-NMR spectra of IV, the signals were similar to those of III, except for the absence of a singlet aldehyde proton signal and a trans double bond. The structure of IV was finally confirmed by comparison with an authentic sample.

Compound V was obtained as a yellow needle and showed a molecular ion peak at m/z 168. The 1H-NMR spectrum exhibited only two singlet signals. One was a methoxy signal at δ 3.83 and the other was a olefinic proton signal at δ 5.86. The 13C-NMR spectrum exhibited five signals, consisting of two carbonyl carbon signals at δ 176.66 and δ 186.83, an oxygenated olefinic carbon signal at δ 157.38, an olefinic carbon signal at δ 107.44 and a methoxy signal at δ 56.48. On the basis of these evidences and of a comparison of the published data (Nishina et al., 1991), V was determined to be 2,6-dimethoxy-1,4-benzoquinone.

The cytotoxicities of the compounds were tested by SRB (Sulforhodamin B) bioassay against five cultured human tumor cells. Compound III showed moderate cytotoxicity, with ED₅₀ values of 9.62 and 5.16 μg/ml against SK-OV-3 and SK-MEL-2, respectively. Compound V exhibited significant cytotoxicity, with ED₅₀ values of 1.46, 1.33, 1.49, 4.22 and 1.82 μg/ml against A549, SK-OV-3, SK-MEL-2, XF498 and HCT15, respectively. However, the other compounds (I, II and IV) showed relatively weak cytotoxicity against the five tested tumor cells.

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