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Phytochemical Constituents of *Artemisia stolonifera*

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Repeated column chromatographic separation of the CH_2Cl_2 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**), (1*S*,7*S*)-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 ED_{50} values ranging from 1.33~4.22 μg/ml.

Key words: *Artemisia stolonifera*, Asteraceae, Cytotoxicity, Simiarenol, (1*S*,7*S*)-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 ^{1}H- and ^{13}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 × 600 mm) and JAIGEL-2H (20 × 600 mm) column. TLC was performed on precoated Kiesel gel 60 F_{254} 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).

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Extraction, separation and purification of compounds

The dried and chopped aerial parts of the plant (2 kg) were extracted with CH_2Cl_2 three times at room temperature. The concentrated CH_2Cl_2 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 [S1-S8 : void volume (400 ml), S1 (500 ml), S2 (80 ml), S3 (220 ml), S4 (850 ml), S5 (1350 ml), S6 (900 ml), S7 (800 ml) and S8 (2600 ml)]. The S4 fraction (1.4 g) was chromatographed with the silica gel column (hexane-CH_2Cl_2-EtOAc=40:60:1) to give four subfractions (S41-S44). The S43 fraction (200 mg) was further subjected to column chromatography over silica gel (hexane-$CHCl_3$-acetone=7:3:1) and purified with recyclic JAI HPLC (GPC column, $CHCl_3$, flow rate 3.5 ml/min) to generate compound **I** (10 mg, t_R=43 min). The S5 fraction (3.4 g) was re-chromatographed over silica gel with hexane-EtOAc (8:1) and purified with a Lobar®-A Si-60 column (hexane-EtOAc=8:1) to generate compound **II** (15 mg). The S6 fraction (1.9 g) was re-chromatographed over silica gel with $CHCl_3$-MeOH (40:1) to yield three subfractions (S61, S62 and S63). The S61 and S62 subfractions were purified with recyclic JAI HPLC (GPC column, $CHCl_3$, flow rate=3.5 ml) to give compounds **III** (20 mg, t_R=56.5 min) and **IV** (12 mg, t_R=58 min), respectively. The S7 fraction (1 g) was further subjected to column chromatography over silica gel ($CHCl_3$-MeOH=30:1) and Sephadex LH-20 (CH_2Cl_2-MeOH=1:1) to produce compound **V** (20 mg).

Simiarenol (I). white powder; $[\alpha]_D$ +46.2° (0.2, $CHCl_3$); mp 213°C; IR ν_{max} (Nujol) cm^{-1}: 3510, 1640, 1460, 1380, 1170, 1050, 840; EIMS m/z (rel. int.): 426 (M^+, 4), 408 (1), 274 (86), 259 (80), 204 (20), 152 (50), 134 (70), 95 (72), 55 (100); ^{1}H-NMR (500 MHz; $CDCl_3$) : δ 0.79 (3H, s, H-28), 0.83 and 0.89 (each 3H, d, *J*=6.5 Hz, H-29, 30), 0.90, 0.93, 1.01, 1.05 and 1.15 (each 3H, s, H-25, 27, 26, 23, 24), 3.48 (1H, br.s, H-3a), 5.62 (1H, br.d, *J*=5.9 Hz, H-6); ^{13}C-NMR (125 MHz; $CDCl_3$) : δ 15.04 (C-27), 15.78 (C-26), 16.10 (C-28), 17.89 (C-25), 18.10 (C-1), 19.95 (C-19), 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-15), 30.81 (C-22), 34.18 (C-11), 34.86 (C-9), 35.45 (C-16), 38.65 (C-13), 39.36 (C-4), 40.86 (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)

(1*S*,7*S*)-1β-Hydroxygermacra-4(15),5,10(14)-triene (II). colorless oil; $[\alpha]_D$ -38.6° (0.2, $CHCl_3$); UV λ_{max} ($CHCl_3$) nm : 243; IR ν_{max} (CCl_4) cm^{-1} : 3300, 1640, 970, 880; EIMS m/z (rel. int.): 220 (M^+, 12), 202 (44), 177 (43), 159 (86), 109 (100), 91 (92), 79 (80); ^{1}H-NMR (500 MHz, $CDCl_3$) : δ 0.84 (3H, d, *J*=6.7 Hz, H-13*), 0.92 (3H, d, *J*=6.7 Hz, H-12*), 1.50-1.82 and 2.64 (6H, m, H-7, 8, 9, 11), 2.06 (2H, m, H-2), 2.21 (1H, ddd, *J*=13.0, 5.5, 2.7 Hz, H-3a), 2.45 (1H, td, *J*=13.0, 4.8 Hz, H-3b), 3.79 (1H, dd, *J*=11.7, 3.9 Hz, H-1), 4.86 (1H, br.s, H-15a), 4.94 (1H, br.s, H-15b), 5.02 (1H, br.s, H-14a), 5.29 (1H, br.s, H-14b), 5.45 (1H, dd, *J*=15.9, 10.3 Hz, H-6), 6.01 (1H, d, *J*=15.9 Hz, H-5); ^{13}C-NMR (125 MHz, $CDCl_3$) : δ 20.49 (C-13*), 20.74 (C-12*), 29.99, 34.54, 36.22, and 36.23 (C-2, 3, 8, 9), 31.80 (C-11), 52.53 (C-7), 76.02 (C-1), 110.54 (C-15), 112.88 (C-14), 129.69 (C-5), 137.94 (C-6), 146.79 (C-4), 153.59 (C-10); *may be interchanged

3'-Methoxy-4'-hydroxy-*trans*-cinnamaldehyde (III). yellow powder; mp 67°C; IR ν_{max} (Nujol) cm^{-1}: 3400, 1660, 1580, 1250; UV λ_{max} ($CHCl_3$) nm : 333, 302 (sh); EIMS m/z (rel. int.): 178 (M^+,100), 161 (35), 147 (60), 135 (68), 107 (44), 84 (35), 77 (34); ^{1}H-NMR (500 MHz, $CDCl_3$) : δ 3.97 (3H, s, OCH_3), 6.03 (1H, s, OH), 6.61 (1H, dd, *J*=15.9, 7.7 Hz, H-2), 6.98 (1H, d, *J*=8.2 Hz, H-5'), 7.08 (1H, d, *J*=1.9 Hz, H-2'), 7.13 (1H,dd, *J*=8.2, 1.9 Hz, H-6'), 7.41 (1H, d, *J*=15.9 Hz, H-3), 9.65 (1H, d, *J* =7.7 Hz, H-1); ^{13}C-NMR (125MHz, $CDCl_3$) : δ 56.05 (OCH_3), 109.53 (C-5'), 114.98 (C-2'), 124.07 (C-6'), 126.51 (C-2), 126.73 (C-1'), 147.00 (C-4'), 148.98 (C-3'), 153.01 (C-3), 193.56 (C-1)

Vanillin (IV). yellow crystal; mp 80°C; ^{1}H-NMR (500 MHz, $CDCl_3$) : δ 3.95 (3H, s, OCH_3), 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); ^{13}C-NMR (125 MHz, $CDCl_3$) : d 56.17 (OCH_3), 108.85 (C-5), 114.41 (C-2), 127.50 (C-6), 130.00 (C-1), 147.18 (C-4), 151.70 (C-3), 190.83 (aldehyde)

2,6-Dimethoxy-1,4-benzoquinone (V). yellow needles; mp 250°C; UV λ_{max} ($CHCl_3$) 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); ^{1}H-NMR (500 MHz, $CDCl_3$) : δ 3.83 (6H, s, OCH_3×2), 5.86 (2H, s, H-3, 5); ^{13}C-NMR (125 MHz, $CDCl_3$) : δ 56.48 (OCH_3×2), 107.44 (C-3, 5), 157.38 (C-2, 6), 176.66 (C-1), 186.83 (C-4)

RESULTS AND DISCUSSION

Compound **I** was obtained as a white amorphorous powder and its molecular formula was deduced to be $C_{30}H_{50}O$ from EIMS (M^+ m/z 426) and ^{13}C-NMR data. The IR spectrum exhibited a hydroxy (3510 cm^{-1}) absorption band. The ^{1}H-NMR spectrum showed an olefinic proton at δ 5.62 (1H, brd, *J*=5.9 Hz, H-6), a carbinol 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 ^{13}C-NMR spectrum exhibited the presence of 30 carbon

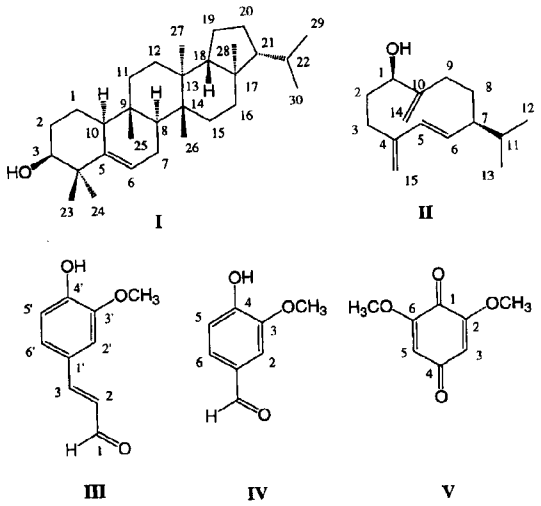

Fig. 1. Structures of the isolated compounds, **I-V**

signals and also showed two olefinic carbon signals at δ122.03 and 142.03, and an oxygenated carbon signal at δ 76.39. The above data were consistent with that for 3-hydroxy-Δ^5-adianene type triterpene. The structure of **I** was determined to be simiarenol 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 ($C_{15}H_{24}O$) 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 ^{1}H-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 ^{13}C-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 (1*S*,7*S*)-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. EIMS and DEPT data established a molecular formula of $C_{10}H_{10}O_3$. The IR spectrum exhibited hydroxy (3400 cm^{-1}) and carbonyl groups (1660 cm^{-1}). The ^{1}H- and ^{13}C-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 ^{1}H-NMR spectrum and δ 193.59 in the ^{13}C-NMR spectrum and a methoxy group at δ 3.97 in the ^{1}H-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 ^{1}H- and ^{13}C-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 ^{1}H-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 ^{13}C-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 ED_{50} values of 9.62 and 5.16 μg/ml against SK-OV-3 and SK-MEL-2, respectively. Compound **V** exhibited significant cytotoxicity, with ED_{50} 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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