

Phytochemical Constituents of *Artemisia stolonifera*

Hak Cheol Kwon, Sang Un Choi¹, and Kang Ro Lee

Natural Products Laboratory, College of Pharmacy, SungKyunKwan University, Suwon, Kyunggi-Do, 440-746, Korea, and ¹Korea Research Institute of Chemical Technology, Daejeon 136-702, Korea

(Received February 2, 2001)

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)-1 β -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 μ g/ml.

Key words: *Artemisia stolonifera*, Asteraceae, Cytotoxicity, Simiarenol, (1S,7S)-1 β -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

¹H- and ¹³C-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 \times 600 mm) and JAIGEL-2H (20 \times 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).

Correspondence to: Prof. Kang Ro Lee, Natural Products Laboratory, College of Pharmacy, SungKyunKwan University, 300 Chonchon-dong, Jangan-ku, Suwon 440-746, Korea
E-mail: krlee@yurim.skku.ac.kr

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; $[\alpha]_D^{20} +46.2^\circ$ (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); $^1\text{H-NMR}$ (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); $^{13}\text{C-NMR}$ (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)

(1S,7S)-1 β -Hydroxygermacra-4(15),5,10(14)-triene (II). colorless oil; $[\alpha]_D^{20} -38.6^\circ$ (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); $^1\text{H-NMR}$ (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); $^{13}\text{C-NMR}$ (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); $^1\text{H-NMR}$ (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); $^{13}\text{C-NMR}$ (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; $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 3.95 (3H, s, OCH_3), 6.20 (1H, s, OH), 7.06 (1H, d, $J=8.4$ Hz, H-5), 7.44 (2H, m, H-2, H-6), 9.85 (1H, s, aldehyde H); $^{13}\text{C-NMR}$ (125 MHz, CDCl_3): δ 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); $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 3.83 (6H, s, $\text{OCH}_3 \times 2$), 5.86 (2H, s, H-3, 5); $^{13}\text{C-NMR}$ (125 MHz, CDCl_3): δ 56.48 ($\text{OCH}_3 \times 2$), 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 amorphous powder and its molecular formula was deduced to be $\text{C}_{30}\text{H}_{50}\text{O}$ from EIMS (M^+ m/z 426) and $^{13}\text{C-NMR}$ data. The IR spectrum exhibited a hydroxy (3510 cm^{-1}) absorption band. The $^1\text{H-NMR}$ 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-3 α), two methyl doublets at δ 0.83 and 0.89, and six methyl singlets at δ 0.79-1.15. The $^{13}\text{C-NMR}$ spectrum exhibited the presence of 30 carbon

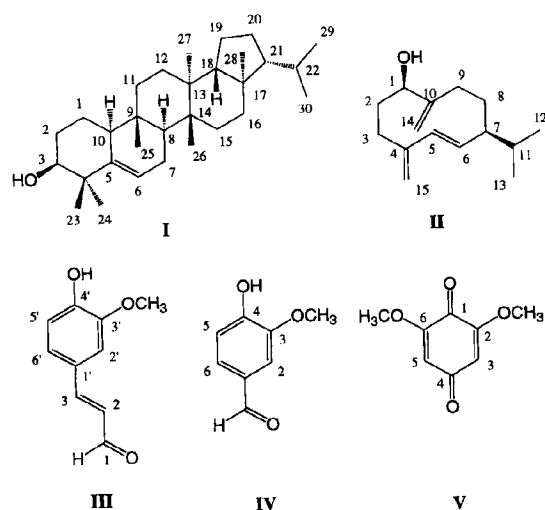


Fig. 1. Structures of the isolated compounds, I-V

signals and also showed two olefinic carbon signals at 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- Δ^5 -adlanene 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 $^1\text{H-NMR}$ 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 $^{13}\text{C-NMR}$ 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*)-1 β -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 $^1\text{H-}$ and $^{13}\text{C-NMR}$ spectra indicated the presence of an aromatic ring, a *trans* double bond [at δ 6.61 (dd, $J=15.9, 7.7\text{ Hz}$) and δ

7.41 (d, $J=15.9\text{ Hz}$)], an aldehyde group at δ 9.65 (d, $J=7.7\text{ Hz}$) in the $^1\text{H-NMR}$ spectrum and δ 193.59 in the $^{13}\text{C-NMR}$ spectrum and a methoxy group at δ 3.97 in the $^1\text{H-NMR}$ 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 $^1\text{H-}$ and $^{13}\text{C-NMR}$ 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 $^1\text{H-NMR}$ 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 $^{13}\text{C-NMR}$ 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 $\mu\text{g/ml}$ 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 $\mu\text{g/ml}$ 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.

ACKNOWLEDGEMENTS

We wish to thank Bang, Eun Jung and Dr. Seo, Jung Ju for the NMR and MS spectra measurements.

REFERENCES

- Bohlmann, F. and Gupta, R. K., Further ineupatorolide-like germacranolides from *Inula cuspidata*, *Phytochemistry*, 21, 157-160 (1982).
- Herath, H.M.T.B., Dassanayake, R.S., Priyadarshani, A.M.A., Silva, S.D., Wannigama, G.P. and Jamie, J., Isoflavonoids and a pterocarpan from *Gliricidia sepium*. *Phytochemistry*, 47, 117-119 (1998).
- Hong, S. W., Kwak, J. H., Kim, D. K., Kwon, H. C., Song, K. W., Lee, K. R., and Zee, O. P., Coumarins from the aerial parts of *Artemisia stolonifera* (Max.) Kom., *Sungkyun Pharm J.*, 7, 7-10 (1995).
- Konovalova, O. A., Rybalko, K. S. and Shreter, A. I.,

- Coumarins from *Artemisia* species, *Khim. Prir. Soedin*, 1, 97 (1976) [Chemical Abstracts, vol. 85 : 30636f (1976)].
- Kwon, H. C., Choi, S. U., and Lee, K. R., Cytotoxic peroxides from *Artemisia stolonifera*. *Arch. Pharm. Res.*, 23, 151-154 (2000).
- Lee, K. R., Hong, S. W., Kwak, J. H., Pyo, S. N., and Zee, O. P., Phenolic constituents from the aerial parts of *Artemisia stolonifera*. *Arch. Pharm. Res.*, 19, 231-234 (1996)
- Lee, T. B., Illustrated Flora of Korea, HyangMunSa, Seoul, p. 760 (1989).
- Nagashima, F., Toyota, M., and Asakawa Y., Terpenoids from some Japanese liverworts, *Phytochemistry*, 29, 2169-2174 (1990).
- Nishina, A., Hasegawa, K., Uchibori, T., Seino, H., and Osawa T., 2,6-Dimethoxy-*p*-benzoquinone as an antibacterial substance in the Bark of *Phyllostachys heterocycla* var. *pubescens*, a species of thick-stemmed bamboo, *J. Agric. Food. Chem.*, 39, 266-269 (1991).
- Song, J. T., Dictionary of botany, HanKuck Do-Seo publishers, Seoul, p. 1034 (1990).
- Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. T., Bokesch, H., Kenney, S., and Boyd, M. R. New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst.* 82, 1107-1112 (1990).
- Tanaka, R. and Matsunaga, S., Triterpene constituents from *Euphorbia supina*, *Phytochemistry*, 27, 3579-3584 (1988).