Cytotoxic Peroxides from *Artemisia stolonifera*

Hak Cheol Kwon, Sang Un Choi[1], and Kang Ro Lee

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

(Received January 21, 2000)

Two sesquiterpene endoperoxides, 1S, 4R, 6R-1, 4-endoperoxy-bisabola-2, 10-diene (**I**), 1R, 4S, 6R-1, 4-endoperoxy-bisabola-2, 10-diene (**II**), and a sesquiterpene hydroperoxide, 1β-hydroperoxygermacra-4 (15), 5, 10 (14)-triene (**III**) were isolated from the aerial parts of *Artemisia stolonifera* (Compositae). Their chemical structures were assigned by spectral evidences. Compounds **I** and **II** exhibited cytotoxicity against five human tumor cell lines with their ED_{50} values ranging from 0.20 to 5.43 μg/ml and from <0.1 to 0.87 μg/ml, respectively.

Key words: *Artemisia stolonifera*, Compositae, Sesquiterpene peroxide, 1S,4R,6R-1,4-Endoperoxy-bisabola-2,10-diene, 1R,4S,6R-1,4-Endoperoxy-bisabola-2,10-diene, 1β-Hydroperoxygermacra-4(15), 5,10(14)-triene, Cytotoxicity

INTRODUCTION

Artemisia stolonifera (Compositae), a perennial herb which is common in Korea, has been used as a folk medicine to treat eye disease, retention of urine and fever (Lee, 1989; Song, 1990). In our previous studies of *A. stolonifera*, we reported the isolation of four coumarins and aromatic compounds (Lee *et al.*, 1996; Hong *et al.*, 1996). Terpene peroxides are very interesting topics because they frequently posess many biological activities such as cytotoxic and antiviral activities (Sam *et al.*, 1998). In peroxide screening of domestic medicinal plants, CH_2Cl_2 extract of *Artemisia stolonifera* showed positive reaction in peroxide test (Lee, 1991). The repeated chromatographic separation of the CH_2Cl_2-soluble peroxide fraction afforded two sesquiterpene endoperoxides (**I** and **II**) and a sesquiterpene hydroperoxide (**III**) (Fig. 1). The present paper describes the isolation, structural characterization and cytotoxicity of two endoperoxides.

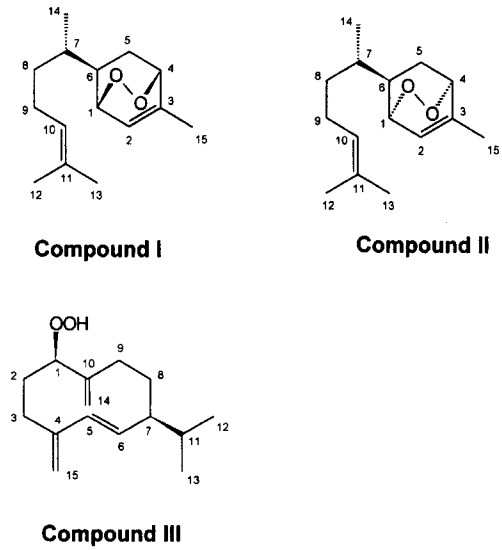

Fig. 1. Structures of compounds I-III

MATERIALS AND METHODS

General experimental procedure

The EIMS spectrum was measured on VG70-VSEQ (VG ANALITICAL, UK). The IR spectrum was measured with a shimadzu IR-435. The ^{1}H- and ^{13}C-NMR spectra were recorded with Bruker AMX-500. The column of LPLC used was Lobar®-A Lichroprep Si-60 (Merck) and pump was DURAMAT 80 (Germany). HPLC was JAI-LC 908 model with UV and Refractive dual detector system and 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). Silica gel for column chromatography was Kiesel gel 60 (70-230 mesh, 230-400 mesh, Merck).

Plant materials

Correspondence to: Natural Products Laboratory, College of Pharmacy, SungKyunKwan University, Suwon, Kyunggi-Do 440-746, Korea
E-mail: krlee@yurim.skku.ac.kr

Artemisia stolonifera was collected in the Kwang-Duck Mt. in Kyunggido in August 1994. A voucher specimen is deposited in the College of Pharmacy at SungKyunKwan University.

Test for cytotoxicity *in vitro*

Sulforhodamin B Bioassay (SRB) was used as a cytotoxicity screening method. Activities of each compounds were tested in several concentration levels against five cultured human tumor cells; A549 (non small cell lung adenocarcinoma), SK-OV-3 (ovarian), SK-MEL-2 (skin melanoma), XF498 (CNS) and HCT15 (colon) *in vitro* (Ryu *et al.*, 1992).

Extraction, separation and purification of compounds

Dried and chopped aerial parts of *Artemisia stolonifera* (2 kg) were extracted with CH_2Cl_2 at room temperature. The concetrated CH_2Cl_2 extract (21 g) was chromatographed through a silica gel column using a gradient solvent system of hexane:EtOAc (10:1-1:1) to yield six fractions designated as AS1-AS6. The peroxide positive AS3 fraction (200 mg) was separated by column chromatography over silica gel eluted with hexane:CH_2Cl_2 (1:2) and purified with JAI recyclic prep. HPLC ($CHCl_3$, flow rate 3.5 ml/min) to afford compound **I** (15 mg, Rt= 48 min). The peroxide positive AS4 fraction (200 mg) was further chromatographed on a silica gel eluted with hexane:CH_2Cl_2 (1:3) and purified with JAI recyclic prep. HPLC ($CHCl_3$, flow rate 3.5 ml/min) to afford compound **II** (10 mg, Rt=47.5 min). The peroxide positive AS6 fraction (170 mg) was further subjected to column chromatography on a silica gel (hexane:$CHCl_3$:acetone= 7:3:1) and purified with Lobar®-A column (hexane: EtOAc=8:1) to afford compound **III** (10 mg).

1S, 4R, 6R-1, 4-endoperoxy-bisabola-2, 10-diene (I), colorless oil; $[\alpha]_D$: -6.7(c 0.2, $CHCl_3$); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2950, 2900, 1650, 1450, 1370, 940, 900; UV $\lambda_{max}^{CHCl_3}$ nm: 239; EIMS *m/z* (rel. int.): 236 (M^+, 0.3), 204 (3), 161 (6), 119 (100), 109 (38), 93 (94); ^{1}H-NMR (500 MHz; $CDCl_3$) δ : 0.99 (3H, d, *J*=6 Hz, H-14), 1.13 (1H, m, H-8a), 1.25 (1H, td, *J*=10.6, 5.0Hz, H-6), 1.57 (1H, m, H-8b), 1.60 (3H, s, H-13), 1.67 (1H, m, H-5a, overlap with H-13), 1.69 (3H, s, H-12), 1.85 (2H, m, H-5b, 7, overlap), 1.92 (1H, m, H-9a), 1.93 (3H, d, *J*=2.0 Hz, H-15), 2.05 (1H, m, H-9b), 4.39 (1H, dt, *J*=4.0, 2.0 Hz, H-4), 4.59 (1H, d, *J*=6.5 Hz, H-1), 5.11 (1H, br.t, *J*=7.0 Hz, H-10), 6.33 (1H, dt, *J*=6.5, 2 Hz, H-2); ^{13}C-NMR (125 MHz; $CDCl_3$) δ: 17.45 (C-14), 17.67 (C-12), 18.58 (C-15), 25.02 (C-9), 25.73 (C-13), 27.38 (C-5), 34.32 (C-8), 34.79 (C-7), 40.02 (C-6), 73.05 (C-1), 76.11 (C-4), 124.62 (C-10), 126.05 (C-2), 131.34 (C-11), 141.28 (C-3)

1R, 4S, 6R-2, 5-endoperoxy-bisabola-2, 10-diene (II), colorless oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2950, 2900, 1650, 1450, 1360, 940, 895; UV $\lambda_{max}^{CHCl_3}$ nm: 239; EIMS m/z (rel. int.): 236 (M^+, 4), 219 (14), 202 (10), 161 (8), 119 (70), 109 (32), 93 (100); ^{1}H-NMR (500 MHz; $CDCl_3$) : δ 0.83 (3H, d, *J*=6.6 Hz, H-14), 1.03 (1H, m, H-5a) 1.11 (2H, m, H-7, 8a, overlap), 1.41 (1H, m, H-8b), 1.61 (3H, s, H-13), 1.70 (3H, d, *J*=1.1 Hz, H-12), 1.88 (1H, m, H-9a), 1.96 (3H, d, *J*=1.8 Hz, H-15), 2.07 (2H, m, H-6, 9b, overlap), 2.35 (1H, ddd, *J*=13.1, 8.8, 4.3 Hz H-5b), 4.45 (1H, dd, *J*=4.3, 1.8 Hz, H-4), 4.63 (1H, m, H-1), 5.07 (1H, br.t, *J*=7.0 Hz, H-10), 6.19 (1H, dt, *J*=6.1, 1.8 Hz, H-2); ^{13}C-NMR (125 MHz; $CDCl_3$) : δ 16.10 (C-14), 17.70 (C-12), 18.57 (C-15), 24.86 (C-9), 25.72 (C-13), 28.39 (C-5), 34.03 (C-8), 36.75 (C-7), 40.09 (C-6), 73.93 (C-1), 75.71 (C-4), 122.97 (C-2), 124.37 (C-10), 131.66 (C-11), 142.04 (C-3)

1S, 7S-1β-hydroperoxygermacra-4 (15), 5, 10 (14)-triene (III), colorless oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3500, 970, 880; UV $\lambda_{max}^{CHCl_3}$ nm: 245; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.83 (3H, d, *J*=6.7 Hz, H-12), 0.92 (3H, d, *J*=6.7 Hz, H-13), 1.5-1.86 and 2.62 (6H, m, H-7, 8, 9, 11), 2.05 (2H, m, H-2), 2.27 (1H, ddd, *J*=13.0, 5.5, 2.6 Hz, H-3a), 2.46 (1H, td, *J*=13.0, 5.0 Hz, H-3b), 4.15 (1H, dd, *J*=11.8, 3.6 Hz, H-1), 4.89 (1H, br.s, H-15a), 4.97 (1H, br.s, H-15b), 5.21 (1H, br.s, H-14a), 5.34 (1H, br.s, H-14b), 5.46 (1H, dd, *J*=15.8, 10.4 Hz, H-6), 6.04 (1H, d, *J*=15.8 Hz, H-5), 7.52 (1H, s, OOH); ^{13}C-NMR (125 MHz, $CDCl_3$) : δ 20.48 (C-12), 20.74 (C-13), 29.30, 30.69, 35.57, and 36.52 (C-2, 3, 8, 9), 31.90 (C-11), 52.69 (C-7), 89.96 (C-1), 113.20 (C-15), 114.58 (C-14), 129.63 (C-5), 138.06 (C-6), 146.35 (C-4), 147.97 (C-10)

RESULTS AND DISCUSSION

Compound **I** was obtained as colorless oil and slowly became blue color in peroxide reagent on TLC plate, suggesting an endo-peroxide (Lee, 1991). Compound **I** showed a molecular ion peak at m/z 236 in EIMS. From the EIMS, ^{1}H-NMR (24H) and ^{13}C-NMR (15C) spectra, the molecular formula was deduced to be $C_{15}H_{24}O_2$. The formula showed four unsaturated bonds equivalents. The ^{13}C-NMR spectrum showed four olefinic carbon signals at δ124.62, δ126.05, δ131.34, δ141.28 and two oxygenated carbon signals at δ73.05 and δ76.11. The ^{1}H-NMR spectrum showed two olefinic protons (δ5.11 and δ6.33), two oxygenated methines (δ4.39 and δ4.59), three methyl singlets (δ1.60, δ1.69 and δ1.93) and a methyl doublet (δ0.99). These data suggested a bisabolane endoperoxide skeleton for compound **I**. ^{1}H-^{1}H COSY spectrum showed correlations between two methyl singlets (δ1.60 and δ1.69) and an olefinic proton (δ5.11), between a methyl singlet (δ1.93) and an olefinic proton (δ6.33), and between an oxygenated methine (δ4.39) and an olefinic proton (δ6.33), respectively. Based on the above considerations and the comparison of the data in

the previous papers (Jang *et al.*, 1993, Sy *et al.*, 1997), the gross structure **I** of was assigned as 1, 4-endoperoxy-bisabola-2, 10-diene.

IR, UV and NMR spectra of compound **II** were almost same as those of compound **I**. The diffenences between and were chemical shifts of H-5, H-6 and H-7 protons in the ^{1}H-NMR spectrum. H-5 protons of compound **I** appeared at δ1.67 and δ1.85 and **II** at δ1.03 and δ2.35, respectively. H-6 proton in an α-endoperoxy group was strongly deshielded and H-7 proton was strongly shielded as compared with β-endoperoxy structure (Sy *et al.*, 1997). These observations indicated that the endoperoxy group in compound **II** was to be α-oriented, and in compound **I** to be β-oriented. Based on the analysis of the NMR spectral data and the comparison of the data in the previous papers (Sy *et al.*, 1997), the structure of **I** was assigned as 1S, 4R, 6R-1, 4-endoperoxy-bisabola-2, 10-diene, and **II** as 1R, 4S, 6R-1, 4-endoperoxy-bisabola-2, 10-diene. The NMR spectral and physical data of compounds **I** and **II** were in good agreement with those reported in the previous paper (Sy *et al.*, 1997). Compounds **I** and **II** were also reported from *Senecio selloi*, and *Eupatorium rufescens* (Ruecker *et al.*, 1996). Compound **II** was also previously isolated from *Artemisia selengensis* in our group (Jang *et al.*, 1993), but the stereochemistry of the endoperoxy group was not determined.

Compound **III** was obtained as colorless oil and positive with peroxide reagent (Lee, 1991). The IR spectrum showed the presence of an OOH group at 3500 cm^{-1}. The molecular ion peak in EI-MS was not detected. The ^{1}H-NMR spectrum showed two secondary methyl groups (δ0.83 and δ0.92), an oxygenated methine proton (δ4.15), two olefinic protons (δ5.46 and δ6.04), four exomethylene protons (δ4.89, δ4.97, δ5.21 and δ5.34), and a hydroperoxy proton (δ7.51). The ^{13}C-NMR spectrum exhibited the presence of 15 carbon signals that composed of six olefinic carbon signals at δ113.20, δ114.58, δ129.63, δ138.06, δ146.35 and δ147.97, one oxygenated carbon signal at 89.96, and eight aliphatic signals at δ20.48, δ20.74, δ29.30, δ30.69, δ35.57, δ36.52, δ31.90 and δ52.69. These spectral data suggested that **III** was a sesquiterpene with a secondary hydroperoxy group. Based on the available chemical structures of the hydroperoxides (Bohlmann *et al.*, 1982) and NMR spectral data, the structure of compound **III** was determined as 1S, 7S-1β-hydroperoxygermacra-4 (15), 5, 10 (14)-triene. The NMR spectral and physical data of compound **III** were in good agreement with those reported in the previous paper (Bohlmann *et al.*, 1982).

The *in vitro* cytotoxicity of compounds **I** and **II** against cultured human tumor cell lines, A549 (non small cell lung adenocarcinoma), SK-OV-3 (ovarian), SK-MEL-2 (skin melanoma), XF498 (CNS) and HCT15 (colon), was studied, and the results are shown in Table **I**. Compounds **I** and **II** exhibited potent cytotoxicity against five human tumor cell lines with their ED_{50} values ranging from 0.20 to 5.43 μg/ml and from <0.1 to 0.87 μg/ml, respectively. These data shows that compound **II** showed very strong cytotoxic activity against SK-OV-3 (colon cacer cells) (ED_{50}: <0.1 μg/ml). The cytotoxicity of compound **III** was not tested, as it was very unstable and left in only trace quantities.

Table I. Cytotoxicity of compounds **I** and **II**

	ED_{50} values*				
cancer cell line	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
I	5.43	0.24	1.20	2.65	0.29
II	0.87	<0.1	0.19	0.32	0.14
doxorubicin	0.12	0.13	0.11	0.23	2.40

*ED_{50} value of compounds against each cancer cell line, which was defined as a concentration (μg/ml) that caused 50 % inhibition of cell growth *in vitro*.

ACKNOWLEDGEMENTS

This paper was supported by Faculty Research Fund, SungKyunKwan University, 1998. We wish to thank Bang, Eun Jung and Seo, Jung Ju for the measurements of NMR and MS spectra.

REFERENCES

Bohlmann, F. and Gupta, R. K., Eremophilene and germacrene derivatives from *Senecio glanduloso-pilosus*. *Phytochemistry*, 17, 2595-2597 (1982).

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*. *Sungkyun Pharm. J.*, 7, 7-10 (1996).

Jang, W. Y. and Lee, K. R., A new endoperoxide from *Artemisia selengensis*. *Korean J. Pharmacogn.*, 24, 107-110 (1993).

Lee, T. B., Illustrated Flora of Korea. HyangMunSa, Seoul, p. 760 (1989).

Lee, K. R., Peroxide constituents in the natural product research. *Kor. J. Pharmacogn.*, 22(3), 145-155 (1991).

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).

Ruecker, G., Schenkel, E. P., Manns, D., Mayer, R., Heiden, K. and Heinzmann, M., Sesquiterpene peroxides from *Senecio selloi* and *Eupatorium rufescens*. *Planta Med.*, 62, 565-566 (1996).

Ryu, S. Y., Lee, C. K., Lee, C. O., Kim, H. S. and Zee, O. P., Antiviral triterpenes from *Prunella vulgaris*. *Arch. Pharm. Res.*, 15, 242-245 (1992).

Sam S., Frederick A.V., Thomas H.C. and Phillip C., Isolation and cytotoxic evaluation of marine sponge-

derived norterpene peroxides. *J. Nat. Prod.*, 61, 241-247 (1988).

Song, J. T., Dictionary of botany. HanKuck Do-Seo publishers, Seoul, p. 1034 (1990).

Sy, L. K. and Brown, G. D., Oxygenated bisabolanes from *Alpinia densibracteata*. *Phytochemistry*, 45, 537-544 (1997).