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Cytotoxic Terpene Hydroperoxides from the Aerial Parts of *Aster spathulifolius*

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Three new sesquiterpene hydroperoxides, 1-[3-(2-hydroperoxy-3-methylbut-3-en)-4-hydroxyphenyl]ethanone (**2**), 7β-hydroperoxy-eudesma-11-en-4-ol (**3**), and 7α-hydroperoxymanool (**4**), together with three known compounds, germacrone (**1**), *ent*-germacra-4(15),5,10(14)-trien-1α-ol (**5**) and teucdiol A (**6**) were isolated from the aerial parts of *Aster spathulifolius* (Compositae). Their structures were characterized using chemical and spectroscopic methods. The isolated compounds were tested for their cytotoxicity against five human tumor cell lines *in vitro* using a SRB method. The two new compounds, **3** and **4**, showed moderate cytotoxicity against human cancer cells with ED_{50} values ranging from 0.24 to 13.27 μg/mL.

Key words: *Aster spathulifolius*, Compositae, Hydroperoxide, Cytotoxicity

INTRODUCTION

Aster spathulifolius (Compositae) is distributed along the east coast of South Korea, and its aerial parts have been used in Korean traditional medicine to treat asthma and diuresis (Lee, 1979). Diterpenes and diterpene glycosides have been reported from this plant (Lee *et al.*, 2005; Uchio *et al.*, 1979, 1980). As part of an ongoing study of this plant, three new sesquiterpene hydroperoxides (**2-4**), together with three known compounds (**1**, **5**, and **6**) were isolated from the MeOH extract of this plant. The isolated compounds were tested for their cytotoxicity against five human tumor cell lines *in vitro* using the SRB bioassay method. This study deals with the structural characterization and cytotoxic activity of the isolated compounds.

MATERIALS AND METHODS

General experimental procedure

Mps: uncorr. NMR: Bruker AMX 500 and Varian UNITY INOVA 500. IR: in CCl_4, Nicolet model 205 FT-IR spectrophotometer. MS: VG70-VSEQ mass spectrometer. Column chromatography : Silica gel 60 (Merck, 70230 mesh and 230400 mesh), Licroprep. RP-18 (Merck) and Sephadex LH-20. TLC: Merck precoated Si gel F254 plates and RP-18 F254s plates. LPLC: Merck Lichroprep Lobar®-A Si 60 (240×10 mm)

Plant materials

A. spathulifolius (Compositae) was collected from Jeju island in August 2001. A voucher specimen (SKK-01-020) was deposited at the Herbarium of the College of Pharmacy, Sungkyunkwan University.

Cytotoxicity test

The sulforhodamine B (SRB) method was used to evaluate the cytotoxicity. The activity of the compounds at several different concentrations was tested against five cultured human tumor cell lines *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 *Aster spathulifolius*

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(8.3 kg) were extracted three times with MeOH at room temperature and evaporated *in vacuo*. The resulting MeOH extract (300 g) was solvent partitioned to give hexane (32 g), CH_2Cl_2 (20 g), EtOAc (8 g) and BuOH (30 g) soluble fractions. The hexane soluble fraction (32 g) was chromatographed over a silica gel column using a gradient solvent system with hexane : EtOAc (10:1~0:1) to give four subfractions (S1~S4). Subfraction S1 (12 g) was chromatographed over a silica gel column eluting with hexane:EtOAc (10:1) to give three subfractions (S11~S13). Subfraction S12 (5 g) was purified with a Sephadex LH-20 column (CH_2Cl_2: MeOH = 1:1) to yield compound **1** (500 mg). Subfraction S2 (6.9 g) was chromatographed with a silica gel column (hexane:EtOAc = 7:1) to give two subfractions (S21 and S22). Subfraction S22 (3 g) was chromatographed over a silica gel column (hexane:EtOAc = 5:1) to give four subfractions (S221~S224). Subfraction S223 (730 mg) was purified using RP-18 Lobar®-A (80% MeCN) and silica gel columns (hexane :EtOAc = 7:1) to yield compound **2** (7 mg). Subfraction S3 (6.5 g) was chromatographed over a silica gel column (hexane:EtOAc = 3:1) to give three subfractions (S31~S33). Subfraction S31 (3 g) was further purified with a Sephadex LH-20 column (CH_2Cl_2:MeOH = 1:1) to give three subfractions (S311~S313). Subfraction S313 (300 mg) was chromatographed with a silica gel Lobar®-A column (hexane:EtOAc = 3:1) to yield compounds **3** (20 mg) and **4** (5 mg). Subfraction S32 (1.5 g) was further purified with a Sephadex LH-20 column (CH_2Cl_2:MeOH = 1:1) to give two subfractions (S321 and S322). Subfraction S322 (700 mg) was purified over a silica gel Lobar-A column (hexane:EtOAc = 3:1) to yield compound **5** (25 mg). Subfraction S4 (4 g) was chromatographed over a silica gel (hexane:EtOAc = 1:1) to give three subfractions (S41 and S42). Subfraction S42 (2.4 g) was chromatographed over a silica gel column (CH_2Cl_2 : MeOH = 30:1) to give three subfractions (S421~S423). Subfraction S423 (600 mg) was purified over a silica gel Lobar-A column (hexane:EtOAc = 1:1) and RP-18 Lobar®-A column (85% MeOH) to yield compound **6** (10 mg).

Germacrone (1)

Colorless needles, mp 50-52°C, EIMS *m/z* (ret. int) : 218 (M^+, 100), 203 (11), 175 (28), 135 (76), 121 (28), 107 (80), 91 (23), 67 (28), 53 (17); ^{1}H-NMR ($CDCl_3$, 500 MHz) : δ 1.43 (3H, s, H-14), 1.61 (3H, s, H-15), 1.71 (3H, s, H-12), 1.76 (3H, s, H-13), 2.0-2.4 (4H, m, H-2, H-3), 2.86 (1H, dd, *J* = 11.5, 13.5 Hz, H-6'), 2.95 (1H, d, *J* = 10.5 Hz, H-9'), 2.97 (1H, dd, *J* = 4.0 and 13.5 Hz, H-6'), 3.41 (1H, d, *J* = 10.5 Hz, H-9'), 4.71 (1H, dd, *J* = 4.0 and 11.5 Hz, H-5), 4.99 (1H, dd, *J* = 3.5 and 12.2 Hz, H-1); ^{13}C-NMR ($CDCl_3$, 125 MHz) : δ 132.2 (C-1), 24.0 (C-2), 37.9 (C-3), 126.8 (C-4), 125.5 (C-5), 28.9 (C-6), 129.0 (C-7), 207.2 (C-8), 55.9 (C-9), 134.9 (C-10), 137 (C-11), 19.8 (C-12), 22.2 (C-13), 15.5 (C-14), 16.7 (C-15).

1-[3-(2-Hydroperoxy-3-methylbut-3-en)-4-hydroxyphenyl] ethanone (2)

Colorless oil, ESIMS *m/z* 236 $[M]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz) : δ 1.78 (3H, br. s, H-5'), 2.56 (3H, s, H-8), 3.08 (1H, dd, *J* = 9.5, 15.5 Hz, H-1'), 3.40 (1H, dd, *J* = 9.5, 18.5 Hz, H-1'), 4.96 (1H, br. d, *J* = 1.5 Hz, H-4'), 5.11 (1H, br. s, H-4'), 5.29 (1H, dd, *J* = 15.5, 18.5 Hz, H-2'), 6.84 (1H, d, *J* = 8.5 Hz, H-5), 7.82 (1H, dd, *J* = 2.0, 8.5 Hz, H-6), 7.84 (1H, d, *J* = 2.0 Hz, H-2); ^{13}C-NMR ($CDCl_3$, 125 MHz,) : δ 127.6 (C-3), 164.2 (C-4), 109.3 (C-5), 125.7 (C-6), 112.8 (C-1), 130.8 (C-2), 196.8 (C-7), 26.6 (C-8), 34.2 (C-1'), 87.2 (C-2'), 143.6 (C-3'), 112.8 (C-4'), 17.3 (C-5').

7β-Hydroperoxy eudesma-11-en-4-ol (3)

Colorless oil, ESIMS *m/z*: 254 $[M]^+$; IR ($CHCl_3$) ν_{max}^{neat} cm^{-1} ν = 3430~3515, 1632, 1447, 1270; ^{1}H-NMR ($CDCl_3$, 500 MHz) : δ 0.92 (3H, s, H-14), 1.06 (3H, s, H-15), 1.83 (3H, br. s, H-13), 3.12 (1H, m, H-5), 4.99 (1H, br. s, H-12), 5.05 (1H, br. s, H-12); ^{13}C-NMR ($CDCl_3$, 125 MHz) : δ 40.6 (C-1), 19.7 (C-2), 44.5 (C-3), 75.7 (C-4), 47.7 (C-5), 40.1 (C-6), 85.6 (C-7), 26.1 (C-8), 27.9 (C-9), 36.4 (C-10), 148.7 (C-11), 111.6 (C-12), 18.8 (C-13), 18.3 (C-14), 22.7 (C-15).

7α-Hydroperoxymanool (4)

Colorless oil, $[\alpha]_D^{20}$ -21.1° (*c* 0.02, $CHCl_3$); IR (neat) ν_{max} 3421, 1622, 1375 cm^{-1}; ESIMS *m/z* 323 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 0.67 (3H, s, H-20), 0.79 (3H, s, H-19), 0.87 (3H, s, H-18), 1.30 (3H, s, H-16), 4.53 (1H, br. t, *J* = 3.0 Hz, H-7), 4.84 (1H, br. s, H-17), 5.20 (1H, br. s, H-17), 5.14 (1H, dd, *J* = 1.0, 11.0 Hz, H-15), 5.27 (1H, dd, *J* = 1.0, 17.5 Hz, H-15), 5.94 (1H, dd, *J* = 11.0, 17.5 Hz, H-14); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 38.6 (C-1), 18.1 (C-2), 42.3 (C-3), 33.4 (C-4), 48.9 (C-5), 30.0 (C-6), 87.1 (C-7), 146.0 (C-8), 50.0 (C-9), 38.8 (C-10), 19.6 (C-11), 39.9 (C-12), 75.1 (C-13), 144.5 (C-14), 113.2 (C-15), 27.9 (C-16), 112.3 (C-17), 31.8 (C-18), 21.4 (C-19), 13.5 (C-20).

***ent*-Germacra-4(15),5,10(14)-trien-1α-ol (5)**

Colorless oil, IR ($CHCl_3$) ν_{max}^{neat} cm^{-1} ν = 3415 (OH), 1680; EIMS *m/z* (ret. Int) : 220 (M^+, 22), 205 (45), 177 (39), 161 (39), 135 (50), 123 (77), 95 (82), 81 (100), 55 (75); ^{1}H-NMR ($CDCl_3$, 500 MHz) : δ 0.81 (3H, d, *J* = 7.0 Hz, H-13), 0.89 (3H, d, *J* = 7.0 Hz, H-12), 1.36 (1H, m, H-11), 1.45~2.03 (each 8H, m, H- 2, 3, 7, 8, and 9), 2.40 (1H, br. dd, *J* = 4.5, 13.0 Hz, H-9), 3.78 (1H, m, H-1), 4.84 (1H, s, H-15), 4.95 (1H, s, H-15), 5.01 (1H, s, H-14), 5.27 (1H, s, H-14), 5.40 (1H, dd, *J* = 10.5 , 16.0 Hz, H-6), 5.97 (1H, d, *J*=16.0 Hz, H-5); ^{13}C-NMR ($CDCl_3$, 125 MHz) : δ 76.1 (C-1), 36.9 (C-2), 30.5 (C-3), 147.4 (C-4), 130.3 (C-5), 138.6

(C-6), 53.2 (C-7), 36.8 (C- 8), 35.2 (C-9), 154.1 (C-10), 32.5 (C-11), 21.2 (C-12), 21.4 (C-13), 111.2 (C-14), 113.6 (C-15).

Teucdiol A (6)

Colorless oil, IR ν_{max}^{neat} cm^{-1} = 3412, 1647, 1450, 1383, 1188; EIMS *m/z* (ret. Int) : 238 (M^+, 23), 205 (18), 191 (23), 187 (15), 135 (33), 123 (100), 107 (34), 98 (30), 83 (46), 69 (41), 55 (38); ^{1}H-NMR ($CDCl_3$, 500 MHz) : δ 0.95 (3H, s), 1.11 (3H, s), 1.81 (3H, br. s), 2.22 (1H, dt, *J* = 2.0, 12.0 Hz, H-5), 5.01 (1H, t, *J* = 0.5 Hz), 5.09 (1H, br. s); ^{13}C-NMR (125 MHz, $CDCl_3$) : δ 40.7 (C-1), 20.2 (C-2), 43.3 (C-3), 75.3 (C-4), 49.0 (C-5), 42.4 (C-6), 71.9 (C-7), 31.3 (C-8), 32.2 (C-9), 34.7 (C-10), 147.0 (C-11), 113.3 (C-12), 18.6 (C-13), 18.7 (C-14), 22.6 (C-15).

Reduction of hydroperoxides (3 and 4)

7β-Hydroperoxy eudesma-11-en-4-ol (**3**, 2 mg) in 1 mL of CH_2Cl_2 was stirred with triphenylphosphine for 10 min at 35°C (Kato *et al.*, 1996). The mixture was evaporated *in vacuo* and subjected to silica gel (1g) column chromatography with *n*-hexane : EtOAc (1:1) to give 1mg of teucdiol A, which was identified by comparison of ^{1}H-NMR data of the compound **6** (teucdiol A). Treatment of 7α-hydroperoxymanool (**4**, 2 mg) with 5 mL of $CHCl_3$ and triphenylphosphine (3 mg) gives 1 mg of an 7α-hydroxymanool, which was identified by comparison of ^{1}H-NMR spectrum data (Cambie *et al.*, 1969).

7α-Hydroxymanool : colorless oil; $[\alpha]_D^{20}$ -9.1° (*c* 0.02, $CHCl_3$); EIMS *m/z* 286 $[M]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 0.67 (3H, s, H-20), 0.82 (3H, s, H-19), 0.90 (3H, s, H-18), 1.29 (3H, s, H-16), 4.38 (1H, br. s, H-17), 4.65 (1H, br. s, H-17), 5.05 (1H, br. s, H-7), 5.08 (1H, dd, *J* = 1.0, 11.0 Hz, H-15), 5.23 (1H, dd, *J* = 1.0, 17.5 Hz, H-15), 5.93 (1H, dd, *J* = 11.0, 17.5 Hz, H-14).

RESULTS AND DISCUSSION

Compounds **1**, **5**, and **6** were identified from a comparison of the spectral data with that reported in the literature as germacrone (Matsuda *et al.*, 2001; Wang & Wang, 2001), *ent*-germacra-4(15),5,10(14)-trien-1α-ol (Bohlmann *et al.*, 1982; Nagashima *et al.*, 1990), and teucdiol A (Fraga *et al.*, 1993), respectively.

Compound **2** was obtained as a colorless oil and tested positive to the peroxide reagent (Lee, 1991). The ESIMS spectrum of compound **2** showed a molecular ion peak at *m/z* 236. The ^{1}H-NMR spectrum showed the presence of two methyl groups at δ 1.78 and 2.56 (each 3H, s), an exomethylene group at δ 4.96 (1H, br. d, *J* = 1.5 Hz) and 5.11 (1H, br. s), aromatic protons at δ 6.84 (1H, d, *J* = 8.5 Hz), 7.82 (1H, dd, *J* = 2.0, 8.5 Hz) and 7.84 (1H, d, *J* = 2.0 Hz), and an oxygenated methine proton at δ 5.29 (1H, dd, *J* = 15.5, 18.5 Hz). The ^{13}C-NMR spectrum showed 13 carbon signals, which consisting of two methyl groups at d 17.3 and 26.6, eight olefinic carbons at δ 109.3, 112.8 (x2), 125.7, 127.6, 130.8, 143.6 and 164.2, a carbonyl carbon at d 196.8 and an peroxygenated carbon at d 87.2. The location of the hydroperoxy group was determined by the HMBC data to be at C-2', which showed a correlation between H-2' and C-4' and 5' (Fig. 2). An analysis of the HMBC and HMQC spectra led to the assignment of all the protons and carbon signals. Based on the above data and the reported chemical structures of the phenolic derivatives from *Artemisia glutinosa* (Gonzalez *et al.*, 1983), the structure of compound **2** was determined to be 1-[3-(2-hydroperoxy-3-methylbut-3-en)-4-hydroxyphenyl] ethanone. The stereochemistry of the hydroperoxy group at C-2' was not characterized because of the small sample size.

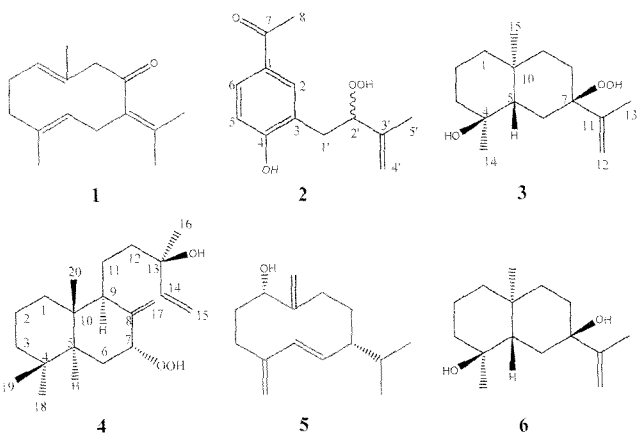

Fig. 1. Structures of compounds **1-6**

Compound **3** was obtained as a colorless oil and tested positive to the peroxide reagent (Lee, 1991). The molecular formula was determined to be $C_{15}H_{26}O_3$ from the ^{1}H-, ^{13}C-NMR and ESIMS data. The IR spectrum of compound **3** showed the presence of a hydroxyl group at 3430~3515 cm^{-1}. The ^{1}H-NMR spectrum showed an isopropylidene group at δ 4.99 (1H, br. s), 5.05 (1H, br. s) and 1.83 (3H, br. s), and two methyl groups at δ 0.92 (3H, s) and 1.06 (3H, s). The ^{13}C-NMR spectrum showed 15 carbon signals including two olefinic crabons (δ 111.6 and 148.7), three methyl carbons (δ 18.3, 18.8 and 22.7), and two oxygenated

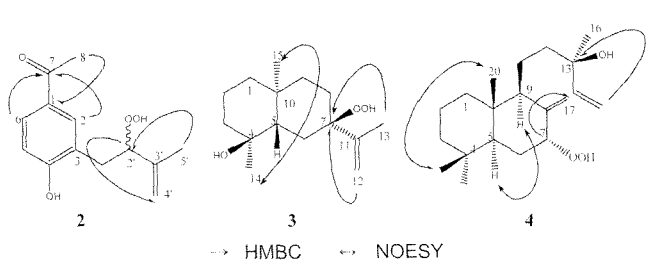

Fig. 2. Selected HMBC and NOESY correlations of **2-4**

carbons (δ 75.7 and 85.6). The ^{1}H- and ^{13}C- NMR spectra of compound **3** were almost same as those of teucdiol A (**6**), which was isolated from *Teucrium heterophyllum* (Fraga *et al.*, 1993). The major difference was the chemical shift of C-7 in the ^{13}C-NMR spectrum, which was shifted downfield by 14 ppm compared with the C-7 in teucdiol A (**6**). The reduction of compound **3** with triphenylphosphine yielded teucdiol A, which was identified by a comparison of the ^{1}H-NMR data. Thus, the structure of compound **3** was determined to be 7β-hydroperoxy-eudesma-11-en-4-ol.

Compound **4** was obtained as a colorless oil and tested positive to the peroxide reagent (Lee, 1991). The molecular formula was assigned as $C_{20}H_{34}O_3$ based on the molecular ion peak $[M+H]^+$ at *m/z* 323 in the ESIMS, ^{1}H- and ^{13}C-NMR (20 C) spectral data. The IR spectrum showed the presence of a hydroxyl group at 3421 cm^{-1}. The ^{1}H-NMR spectrum showed the presence of four methyl groups at δ 0.67, 0.79, 0.87 and 1.30 (each 3H, s), a carbinol proton at δ 4.53 (1H, br. t, *J* = 3.0 Hz), an exomethylene group at δ 4.84 (1H, br. s) and 5.20 (1H, br. s), and an ABM coupling system of three olefinic protons at δ 5.14 (1H, dd, *J* = 1.0, 11.0 Hz), 5.27 (1H, dd, *J* = 1.0, 17.5 Hz) and 5.94 (1H, dd, *J* = 11.0, 17.5 Hz). The ^{13}C-NMR spectrum showed 20 carbon signals, which contained four olefinic carbons at δ 112.3, 113.2, 144.5 and 146.0, and two oxygenated carbon at δ 75.1 and 87.1. The ^{1}H- and ^{13}C-NMR spectra of compound **4** were similar to those of labda-8(17),14-diene-7α,13-diol (7α-hydroxymanool) (Cambie *et al.*, 1969). The major differences were the chemical shift of H-7 in the ^{1}H-NMR spectrum (compound **4** : δ 4.53, 7α-hydroxymanool : δ 5.05) and C-7 in the ^{13}C-NMR spectrum (compound **4** : δ 87.1, 7α-hydroxymanool : δ 73.9). The reduction of compound **4** with triphenylphosphine yielded 7α-hydroxymanool, whose ^{1}H-NMR spectrum was in good agreement with that reported previously (Cambie *et al.*, 1969). An analysis of the HMBC and HMQC spectra led to the assignment of all proton and carbon signals for compound **4**. Therefore, the structure of compound **4** was determined to be 7α-hydroperoxy-labda-8(17),14-dien-13(*R*)-ol (7α-hydroperoxymanool).

Compounds **1-6** were evaluated for their cytotoxic activity against five human tumor cell lines. Compound **3** showed non-specific moderate cytotoxic activity against the human non-small cell lung cancer cells (ED_{50} values; A549, 1.25 μg/mL; SK-OV-3, 13.27 μg/mL; SK-MEL-2, 8.49 μg/mL, XF498, 9.57 μg/mL; HCT15, 8.89 μg/mL, respectively). Compound **4** showed non-specific excellent cytotoxic activity (ED_{50} values; A549, 2.54 μg/mL; SK-OV-3, 1.33 μg/mL; SK-MEL-2, 0.24 μg/mL, XF498, 1.57 μg/mL; HCT15, 1.41 μg/mL, respectively). The other compounds showed little activity against the five human cancer cell lines tested (>10 μg/mL).

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