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Cytotoxic Terpenes and Lignans from the Roots of *Ainsliaea acerifolia*

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The chromatographic separation of the MeOH extract of the roots of *Ainsliaea acerifolia* (Compositae) led to the isolation of six known terpenes and two known lignans. Their structures were identified by spectroscopic methods as mokko lactone (**1**), betulonic acid (**2**), betulinic acid (**3**), zaluzanin C (**4**), 1β-hydroperoxygermacra-4(15),5,10(14)-triene (**5**), pluviatilol (**6**), (+)-syringaresinol (**7**), and glucozaluzanin C (**8**). Compounds **1~4** and **8** showed non-specific significant cytotoxicity against five human tumor cell lines with ED_{50} values ranging from 0.36~5.54 μg/mL.

Key words: *Ainsliaea acerifolia*, Compositae, Sesquiterpene lactone, Triterpenoid, Lignan, Cytotoxicity

INTRODUCTION

Ainsliaea acerifolia (Compositae), a perenial herb, is distributed in the mountain of south Korea. This plant has been used for the treatment of rheumatic arthritis and enteritis in the Chinese folk medicine (Kim *et al.*, 1999). Sesquiterpene lactones and lipid glycerols were reported from this plant (Toshio *et al.*, 1984). We have isolated three sesquiterpene lactones and two glycerols from the aerial parts of this species (Jung *et al.*, 2000). In our continuing study on the biological active compounds of this source, we have investigated the constituents of roots of *A. acerifolia*. As a result, we have isolated six known terpenoids and two known lignans from the hexane and CH_2Cl_2 soluble fractions of the MeOH extract. Their structures were characterized by spectroscopic means. The isolated compounds were tested for cytotoxicity against five human tumor cell lines *in vitro* by SRB assay. This paper describes the isolation, structural characterization and cytotoxicity of the isolated compounds.

MATERIALS AND METHODS

General experimental procedure

Mps: uncorr. Optical rotation: Jasco P-1020 Polarimeter. NMR: Bruker AMX 500 and Varian Unity Inova 500. IR: in CCl4, Nicolet model 205 FT-IR spectrophotometer. MS: VG70-VSEQ mass spectrometer. Column chromatography : Silica gel 60 (Merck, 70~230 mesh and 230~400 mesh), Lichroprep. RP-18 (Merck) and Sephadex LH-20. TLC: Merck precoated Si gel F_{254} plates and RP-18 F_{254s} plates. LPLC: Merck Lichroprep Lobar®-A Si 60 (240×10mm).

Plant materials

The roots of *Ainsliaea acerifolia* (Compositae) were collected at Gangwon province in August, 2003. A voucher specimen (SKK-03-010) was deposited at the herbarium of the College of Pharmacy in Sungkyunkwan University.

Cytotoxicity testing

Sulforhodamin B assay (SRB) was used for cytotoxicity evaluation. The activity of a compound was tested at 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) (Skehan *et al.*, 1990).

Extraction, separation and purification of compounds

The dried and chopped roots of *Ainsliaea acerifolia* (1.8 kg) were extracted with MeOH (10 L) five times at room temperature. The resulting methanol extract (120 g) was

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successively partitioned and evaporated to give *n*-hexane (15 g), CH_2Cl_2 (10 g), EtOAc (10 g) and BuOH (20 g). The *n*-hexane extract (15 g) was chromatographed over a silica gel column using gradient solvent system of *n*-hexane:EtOAc (10:1~0:1) to give seven fractions (AAH-1 ~AAH-7). The fraction AAH-2 (0.7 g) was purified with Sephadex LH-20 (CH_2Cl_2:MeOH=1:1) and Lobar®-A column (*n*-hexane:EtOAc=6:1) to yield **1** (12 mg). The fraction AAH-3 (1.6 g) was chromatographed over a silica gel column eluted with *n*-hexane:EtOAc (5:1) to give three subfractions (AAH-31~AAH-33). The subfraction AAH-31 (300 mg) was purified with Sephadex LH-20 (CH_2Cl_2 : MeOH=1:1) and HPLC (*n*-hexane:EtOAc=5:1) to yield **2** (10 mg). The subfraction AAH-33 (800 mg) was purified with Sephadex LH-20 (CH_2Cl_2:MeOH=1:1) and Lobar,-A column (*n*-hexane:EtOAc=4:1) to yield **3** (10 mg), and **4** (9 mg). The fraction AAH-4 (1.2 g) was purified with Sephadex LH-20 (CH_2Cl_2:MeOH=1:1), Lobar®-A column (*n*-hexane:EtOAc=3:1) and HPLC (*n*-hexane: EtOAc=2:1) to yield **5** (15 mg). The CH_2Cl_2 extract (10 g) was chromatographed over a silica gel column using gradient solvent system of *n*-hexane:EtOAc (3:1~1:1) to give six fractions (AAM-1~AAM-6). The fraction AAM-2 (0.8 g) was purified with Sephadex LH-20 (CH_2Cl_2: MeOH =1:1), RP Lobar®-A column (70% MeOH) and HPLC (*n*-hexane:EtOAc:MeOH=2:2:0.1) to yield **6** (12 mg). The fraction AAM-3 (0.5 g) was purified with Sephadex LH-20 (CH_2Cl_2:MeOH=1:1) and HPLC (*n*-hexane:EtOAc:MeOH= 1:1:0.1) to yield **7** (10 mg). The fraction AAM-4 (0.8 g) was purified with Sephadex LH-20 (CH_2Cl_2:MeOH =1:1), and Lobar,-A column ($CHCl_3$:EtOAc:MeOH=45:20:6) to yield **8** (8 mg).

4(15), 10(14)-Guaiadien-12, 6-olide (mokko lactone) (1)

White powder, $[\alpha]^{20}_D$ +21.2° (c 0.04, $CHCl_3$); mp 37°C; IR ($CHCl_3$) ν_{max} cm^{-1} : 1772 (γ-lactone), 1620 (C=C); EIMS *m/z* (rel. int.) : 232 (M^+, 7), 158 (100), 152 (62), 91 (71), 71 (63), 55 (64); ^{1}H-NMR ($CDCl_3$, 500MHz) : δ 5.21 (1H, d, *J* = 2.0 Hz, H-15a), 5.06 (1H, d, *J* = 2.0 Hz, H-15b), 4.89 (1H, br. s, H-14a), 4.79 (1H, br. s, H-14b), 3.93 (1H, t, *J* = 9.5 Hz, H-6), 2.89 (1H, dt, *J* = 8.0, 4.5 Hz, H-1), 2.81 (1H, br. dd, *J* = 9.5, 8.0 Hz, H-5), 2.49 (3H, m, H-3, 11), 2.22 (1H, dd, *J* = 12.0, 7.0 Hz, H-9), 2.12 (1H, m, H-7), 2.05 (1H, dt, *J* = 12.0, 5.0 Hz, H-9), 1.95 (1H, m, H-2), 1.94 (1H, m, H-8), 1.87 (1H, m, H-2), 1.32 (1H, m, H-8), 1.25 (3H, d, *J* = 7.0 Hz, H-13); ^{13}C-NMR ($CDCl_3$, 125MHz) : δ 179.0 (C-12), 152.0 (C-4), 150.2 (C-10), 112.1 (C-14), 109.5 (C-15), 85.6 (C-6), 52.2 (C-5), 50.1 (C-11), 47.3 (C-1), 42.3 (C-7), 37.9 (C-9), 32.8 (C-3, 8), 30.5 (C-2), 13.5 (C-13).

Betulonic acid (2)

White powder, mp 258°C; EIMS *m/z* (rel. int.) : 454 (M^+, 8), 248 (34), 207 (71), 203 (40), 189 (100); ^{1}H-NMR ($CDCl_3$, 500MHz) : δ 4.74 and 4.61 (each 1H, br. s, H-29), 1.69 (3H, s, H-30), 1.44, 1.08, 1.01, 0.99, 0.97 (each 3H, s); ^{13}C-NMR ($CDCl_3$, 125MHz) : δ 15.3 (C-27), 16.5 (C-26), 16.6 (C-25), 20.0 (C-30), 20.3 (C-6), 21.6 (C-24), 22.0 (C-11), 26.2(C-12), 27.3 (C-23), 30.3 (C-21), 31.2 (C-15), 32.8 (C-16), 34.3 (C-7), 34.8 (C-2), 37.6 (C-22), 37.7 (C-10), 39.2 (C-13), 40.3 (C-1), 41.3 (C-8), 43.2 (C-14), 47.5 (C-19), 48.0 (C-4), 49.9 (C-18), 50.5 (C-9), 55.6 (C-5), 57.0 (C-17), 110.4 (C-29), 150.9 (C-20), 182.1 (C-28), 218.7 (C-3).

Betulinic acid (3)

White powder, mp 282°C; EIMS *m/z* (rel. int.) : 456 (M^+, 30), 438 (12), 411 (6), 248 (45), 228 (58), 207 (66), 203 (38), 189 (100); ^{1}H-NMR (C_5D_5N, 500MHz) : δ 4.94 (1H, d, *J* = 2.0 Hz), 4.77 (1H, s), 3.55 (1H, m, H-3), 1.80, 1.23, 1.08, 1.07, 1.02 and 0.83 (each 3H, s, 6×CH_3); ^{13}C-NMR (C_5D_5N, 125MHz) : δ 15.6. (C-27), 17.1 (C-24), 17.2 (C-25), 16.1 (C-26), 18.3 (C-6), 19.4 (C-30), 20.8 (C-11), 25.5(C-12), 27.4 (C-2), 27.9 (C-23), 29.7 (C-21), 30.5 (C-15), 32.1 (C-16), 34.3 (C-7), 37.0 (C-22), 37.2 (C-10) 38.4 (C-13), 38.7 (C-1), 38.8 (C-4), 42.0 (C-8), 43.6 (C-14), 48.5 (C-18), 50.4 (C-19), 51.5 (C-9), 57.4 (C-5), 58.1 (C-17), 80.5 (C-3), 110.6 (C-29), 152.1 (C-20), 179.6 (C-28).

Zaluzanin C (4)

Colorless oil, $[\alpha]^{20}_D$ +53.7° (c 0.08, $CHCl_3$); IR ($CHCl_3$) ν_{max}^{neat} cm^{-1} ν = 3410, 1771, 1712 and 1653 cm^{-1}; EIMS *m/z* (rel. int.) : 246 (M^+, 60), 228 (31), 218 (26), 200 (29), 175 (28), 150 (48), 105 (63), 91 (100); ^{1}H-NMR ($CDCl_3$, 500MHz) : δ 1.47 (1H, m, H-8), 1.72~1.79 (1H, m, H-2), 2.13 (1H, m, H-9), 2.21~2.32 (2H, m, H-2, 8), 2.49 (1H, ddd, *J* = 6.0, 6.0, 12.0 Hz, H-9), 2.81 (2H, m, H-1, 7), 2.92 (1H, dd, *J* = 9.0, 17.5 Hz, H-5), 4.10 (1H, dd, *J* = 9.0, 9.0 Hz, H-6), 4.55 (1H, br. t, *J* = 7.5 Hz, H-3), 4.94 (1H, s, H-14), 4,99 (1H, br. s, H-14), 5.28 (1H, br. s, H-15), 5.42 (1H, br. s, H-15), 5.48 (1H, d, *J* = 3.0 Hz, H-13), 6.19 (1H, d, *J* = 3.0 Hz, H-13); ^{13}C-NMR ($CDCl_3$, 125MHz) : δ 31.2 (C-8), 34.9 (C-9), 39.8 (C-2), 44.7 (C-1), 46.1 (C-7), 50.6 (C-5), 74.2 (C-3), 84.7 (C-6), 111.8 (C-15), 115.1 (C-14), 121.0 (C-13), 140.5 (C-11), 148.6 (C-10), 153.7 (C-4), 170.6 (C-12).

1β-Hydroperoxygermacra-4(15),5,10(14)-triene (5)

Colorless oil, $[\alpha]^{20}_D$ -43.2° (c 0.06, $CHCl_3$); IR ($CHCl_3$) ν_{max}^{neat} cm^{-1} = 3503, 1641 cm^{-1}; ESIMS *m/z* : 236 $[M]^+$; ^{1}H-NMR ($CDCl_3$, 500MHz) : δ 0.83 (3H, d, *J* = 6.5 Hz, H-12), 0.92 (3H, d, *J* = 6.5 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* = 2.5, 5.5, 13.0 Hz, H-3a), 2.46 (1H, td, *J* = 5.0, 13.0 Hz, H-3b), 4.15 (1H, dd, *J* = 3.5, 12.0 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* = 16.0, 10.5 Hz, H-6), 6.04 (1H, d, *J* = 16.0 Hz, H-5); ^{13}C-NMR ($CDCl_3$, 125MHz) : δ 20.5 (C-12), 20.7 (C-13), 29.3, 30.7, 35.6, and 36.5 (C-2, 3, 8, 9), 31.9 (C-11), 52.7 (C-7), 89.9 (C-1), 113.2 (C-15), 114.6 (C-14), 129.6 (C-5), 138.1 (C-6), 146.4 (C-4), 148.0 (C-10).

Pluviatilol (6)

Colorless gum, $[\alpha]^{20}_D$ + 79.8° (c 0.16, MeOH); EIMS *m/z* (rel. int.) : 356 (M^+, 100), 205 (18), 163 (22), 161 (27), 151 (74), 149 (64), 137 (31), 135 (41), 131 (30), 122 (13); ^{1}H-NMR ($CDCl_3$, 500 MHz) : δ 2.91 (1H, m, H-1), 4.42 (1H, d, *J* = 7.5 Hz, H-2), 3.32 (1H, m, H-4), 3.85 (1H, m, H-4), 3.32 (1H, m, H-5), 4.86 (1H, d, *J* = 6.0 Hz, H-6), 3.85 (1H, dd, *J* = 6.5, 9.5Hz, H-8), 4.13 (1H, dd, *J* = 1.0, 9.5 Hz, H-8), 6.81~6.89 (6H, H-2', 5', 6', 2", 5", 6"), 5.97 (2H, s, OCH_2O), 3.91 (3H, s, OCH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz) : δ 55.2 (C-1), 88.2 (C-2), 71.6 (C-4), 50.9 (C-5), 82.7 (C-6), 70.3 (C-8), 133.0 (C-1'), 109.3 (C-2'), 147.3 (C-3'), 146.0 (C-4'), 114.9 (C-5'), 119.3 (C-6'), 133.7 (C-1"), 107.1 (C-2"), 148.3 (C-3"), 147.4 (C-4"), 108.8 (C-5"), 120.0 (C-6"), 101.7 (OCH_2O), 56.6 (OCH_3).

(+)-Syringaresinol (7)

Colorless needle, mp 180°C; $[\alpha]^{20}_D$ + 6.9° (c 0.02, $CHCl_3$); EIMS *m/z* (rel. int.): 418 (M^+, 100), 235 (10), 210 (16), 193 (29), 181 (69), 167 (58); ^{1}H-NMR ($CDCl_3$, 500 MHz) : δ 3.11 (1H, m, H-1), 4.73 (1H, d, *J* = 4.5 Hz, H-2), 3.89 (1H, m, H-4), 4.30 (1H, dd, *J* = 7.0, 9.0 Hz, H-4), 3.12 (1H, m, H-5), 4.73 (1H, d, *J* = 4.5 Hz, H-6), 3.89 (1H, m, H-8), 4.30 (1H, dd, *J* = 7.0, 9.0 Hz, H-8), 6.58~6.60 (4H, H-2', 6', 2", 6"), 3.90 (3H, s, OCH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz) : δ 55.1 (C-1), 86.8 (C-2), 72.5 (C-4), 55.1 (C-5), 86.8 (C-6), 72.5 (C-8), 132.8 (C-1'), 103.4 (C-2'), 147.9 (C-3'), 135.0 (C-4'), 147.9 (C-5'), 103.4 (C-6'), 132.8 (C-1"), 103.4 (C-2"), 147.9 (C-3"), 135.0 (C-4"), 147.9 (C-5"), 103.4 (C-6"), 57.1 (OCH_3).

Glucozaluzanin C (8)

White powder, mp 103°C; $[\alpha]^{20}_D$ - 15.7° (c 0.04, $CHCl_3$); EIMS *m/z* (rel. int.) : 408 (M^+, 1), 229 (26), 201 (19), 183 (21), 105 (50), 91 (100); ^{1}H-NMR ($CDCl_3$, 500MHz) : δ 1.46 (1H, m, H-8), 1.98 (1H, m, H-2), 2.21 (1H, m, H-9), 2.28 (1H, m, H-8), 2.39 (1H, m, H-2), 2.52 (1H, ddd, *J* = 6.5, 6.5, 13.0 Hz, H-9), 2.80 (1H, br. t, *J* = 10.0 Hz, H-1), 2.89 (1H, m, H-7), 3.01 (1H, dd, *J* = 8.5, 17.5 Hz, H-5), 3.20~3.40 (4H, m, H-2', 4', 6'), 3.67 (1H, dd, *J* = 5.5, 12.0 Hz, H-5'), 3.87 (1H, dd, *J* = 10.0, 10.0 Hz, H-3'), 4.28 (1H, dd, *J* = 9.0, 9.0 Hz, H-6), 4.47 (1H, d, *J* = 7.5 Hz, H-1'), 4.65 (1H, br. dd, *J* = 6.0, 6.0 Hz, H-3), 4.94 (1H, br. s, H-14), 5.01 (1H, br. s, H-14), 5.35 (1H, br. d, *J* = 1.0 Hz, H-15), 5.44 (1H, br. s, H-15), 5.57 (1H, d, *J* = 3.0 Hz, H-13), 6.12 (1H, d, *J* = 3.0 Hz, H-13); ^{13}C-NMR ($CDCl_3$, 125MHz) : δ 31.0 (C-8), 33.9 (C-9), 38.0 (C-2), 45.4 (C-1), 45.8 (C-7), 50.7 (C-5), 62.1 (C-6'), 71.1 (C-4'), 74.3 (C-2'), 77.2 (C-5'), 77.5 (C-3'), 80.7 (C-3), 84.7 (C-6), 102.3 (C-1'), 112.8 (C-15), 114.1 (C-14), 120.0 (C-13), 141.5 (C-11), 149.3 (C-10), 150.2 (C-4), 171.6 (C-12).

RESULTS AND DISCUSSION

Compound **1** was obtained as white powder. The IR spectrum showed a γ-lactone band (1772 cm^{-1}) and double bond (1620 cm^{-1}). From the EI-MS, ^{1}H- and ^{13}C-NMR spectral data, the molecular formula was deduced to be $C_{15}H_{20}O_2$. The signals at δ 3.93 (1H, t, *J* = 9.5 Hz) and 2.05 (1H, dt, *J* = 5.0, 12.0 Hz) was indicative to the presence of a lactone ring, and the ^{1}H-NMR spectrum showed four exomethylene protons at δ 5.21 (1H, d, *J* = 2.0 Hz), 5.06 (1H, d, *J* = 2.0 Hz), 4.89 (1H, br. s), 4.79 (1H, br. s). The ^{13}C-NMR spectrum exhibited 15 carbon signals, consisting of four olefinic carbon signals at δ 109.5, 112.1, 150.2 and 152.0, a carbonyl carbon signal at d 179.0, and an oxygenated carbon signal at δ 85.6. The ^{1}H-NMR and ^{13}C-NMR spectra showed the typical pattern of guaiane-type sesquiterpene lactone (Marco *et al.*, 1993; Marco *et al.*, 1994). Based on the above consideration and the comparison of the data with those in the previous papers (Hikino *et al.*, 1967; Yuuya *et al.*, 1999), the structure of **1** was identified as 4(15), 10(14)-guaiadien-12, 6-olide (mokko lactone).

Compound **2** was obtained as a white powder. The ^{1}H-NMR spectrum showed six methyl groups at δ 0.97, 0.99, 1.01, 1.08, 1.44 and 1.69, a vinylic protons of terminal methylene group at d 4.74 and 4.61 (each 1H, br. s). The ^{13}C-NMR spectrum exhibited the presence of 30 carbon signals, consisting of six methyl signals at δ 15.3, 16.5, 16.6, 20.0, 21.6, 27.3, two olefinic carbon signals at d 110.4 and 150.9, a carboxylic acid carbon signal at δ 182.1, and a ketone signal at δ 218.7. These spectral data suggested that **2** was a triterpenoic acid. Based on the above mentioned data and the reported chemical structures of triterpenes (Mahato & Kundu, 1994), the structure of **2** was determined to be betulonic acid (Gonzalez *et al.*, 1983).

Compound **3** was obtained as a white powder. From the EIMS *(m/z* 456), ^{1}H- and ^{13}C-NMR spectral data of **3**, the molecular formula was deduced to be $C_{30}H_{48}O_3$. The ^{1}H- and ^{13}C-NMR spectra of compound **3** were almost same as those of compound **2**. The differences were the absence of a ketone signal at δ 218.7 (C-3, in **2**) and the presence of hydroxyl group (δ 3.55, H-3 and δ 80.5, C-3) in **3**. Based on the above mentioned data and the reported chemical structures of triterpenes (Mahato & Kundu, 1994), the structure of **3** was determined to be betulinic acid (Kojima *et al.*, 1987).

Compound **4** was obtained as a colorless oil. The EIMS spectrum of **4** showed a molecular ion peak at *m/z* 246. The IR spectrum showed the band of α,β-unsaturated lactone group at 1771 cm^{-1} and the hydroxyl group at 3410 cm^{-1}. The ^{1}H-NMR spectrum showed methine protons of γ-lactone ring at d 2.81 (1H, m) and 4.10 (1H, dd, *J* = 9.0, 9.0 Hz), an oxygenated methine proton at d 4.55 (1H, br. t, *J* = 7.5 Hz), and six exomethylene protons at d 4.94 (1H, s), 4.99 (1H, br. s), 5.28 (1H, br. s), 5.42 (1H, br. s), 5.48 (1H, d, *J* = 3.0 Hz), 6.19 (1H, d, *J* = 3.0 Hz). The ^{13}C-NMR spectrum exhibited 15 carbon signals, consisting of six olefinic carbon signals at δ 111.8, 115.1, 121.0, 140.5, 148.6 and 153.7, a carbonyl carbon signals at δ 170.6, and two oxygenated carbon signals at δ 74.2 and 84.7. These spectral data suggested that **4** was a guaiane sesquiterpene lactone (Li *et al.*, 1989; Singhal *et al.*, 1982; Zdero *et al.*, 1991). Based on the above mentioned data and the reported chemical structures of sesquiterpene lactones (Kisiel, 1983; Marco *et al.*, 1994), the structure of **4** was determined to be zaluzanin C (Ando *et al.*, 1989).

Compound **5** was obtained as colorless oil and positive with peroxide reagent (Lee, 1991). The ESIMS spectrum of **5** showed a molecular ion peak at *m/z* 236. The ^{1}H-NMR spectrum showed two secondary methyl groups at δ 0.83 and 0.92, an oxygenated methine proton at δ 4.15, two olefinic protons at δ 5.46 and 6.04, four exomethylene protons at δ 4.89, 4.97, 5.21 and 5.34. The ^{13}C-NMR spectrum exhibited the presence of 15 carbon signals, composed of six olefinic carbon signals at δ 113.2, 114.6, 129.6, 138.1, 146.4 and 148.0, one oxygenated carbon signal at δ 89.9, and eight aliphatic signals. Based on the reported structures of sesquiterpene hydroperoxides (Bohlmann & Gupta, 1982) and NMR spectral data, the structure of **5** was determined as 1β-hydroperoxygermacra-4(15),5,10(14)-triene (Bohlmann & Gupta, 1982).

Compound **6** was obtained as colorless gum and afforded a molecular ion (M^+) peak at *m/z* 356 in EIMS spectrum. Its ^{1}H-NMR spectrum exhibited signals for six aromatic protons (δ 6.81~6.89), a dioxymethylene group (δ 5.97), a methoxy group (δ 3.91). The ^{13}C-NMR spectrum exhibited the presence of 20 carbon signals, composed of four oxygenated aromatic carbons (δ 146.0, 147.3, 147.4

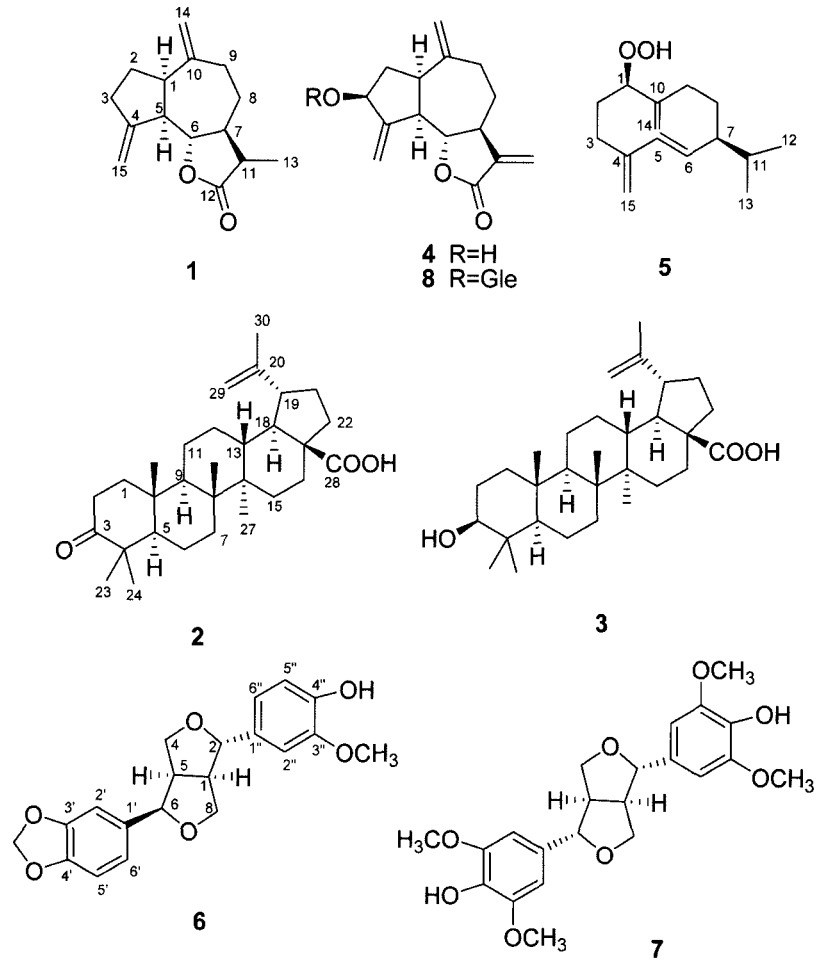

Fig. 1. Structures of compounds **1~8**

and 148.3), a dioxymethylene carbon (δ 101.7), four oxygenated aliphatic carbons (δ 70.3, 71.6, 82.7, and 88.2), and a methoxy group (δ 56.6). Eight signals at d 2.91 (1H, m, H-1), 4.42 (1H, d, *J* = 7.5 Hz, H-2), 3.32 (1H, m, H-4), 3.85 (1H, m, H-4), 3.32 (1H, m, H-5), 4.86 (1H, d, *J* = 6.0 Hz, H-6), 3.85 (1H, dd, *J* = 6.5, 9.5 Hz, H-8), 4.13 (1H, dd, *J* = 1.0, 9.5 Hz, H-8) were indicative of *epi*-furofuran type lignan. Thus, the structure of **6** was characterized to be pluviatilol by spectroscopic data and literatures survey (Banerji & Pal, 1982; Macrae & Towers, 1985). The NMR spectral and physical data of the **6** were in good agreement with those reported in the previous papers (Corrie *et al.*, 1970; Ishii *et al.*, 1983).

Compound **7** was obtained as a white powder and afforded a molecular ion (M^+) peak at *m/z* 418 in EIMS spectrum. The ^{1}H-NMR spectrum exhibited signals for four aromatic protons (δ 6.58~6.60) and four aromatic methoxy groups (δ 3.89, 12H, OCH_3). Eight signals at δ 3.11 (1H, m, H-1), 4.73 (1H, d, *J* = 4.5 Hz, H-2), 3.89 (1H, m, H-4), 4.30 (1H, dd, *J* = 7.0, 9.0 Hz, H-4), 3.12 (1H, m, H-5), 4.73 (1H, d, *J* = 4.5 Hz, H-6), 3.89 (1H, m, H-8), 4.30 (1H, dd, *J* = 7.0, 9.0 Hz, H-8) were indicative of the typical pattern of symmetric furofuran lignan (Macrae & Towers, 1985; Tanaka *et al.*, 1989). Thus, the structure of **7** was characterized to be (+)-syringaresinol by spectroscopic data and comparing its physical and spectroscopic data with those in the published literature (Deyama *et al.*, 1987).

Compound **8** was obtained as a white powder. The ^{1}H- and ^{13}C-NMR spectra of **8** were quite similar to those of **4**. The major difference in the NMR spectra was the presence of sugar signals in **8** [^{1}H-NMR: δ 3.20~3.40 (4H, m, H-2', 4', 6'), 3.67 (1H, dd, *J* = 5.5, 12.0 Hz, H-5'), 3.87 (1H, dd, *J* = 10.0, 10.0 Hz, H-3'), and 4.47 (1H, d, *J* = 7.5 Hz, H-1'); ^{13}C-NMR: δ 62.1 (C-6'), 71.1 (C-4'), 74.3 (C-2'), 77.2 (C-5'), 77.5 (C-3') and 102.3 (C-1')]. Based on the above mentioned data and the reported chemical structures of sesquiterpene lactones (Kisiel, 1983; Marco *et al.*, 1994), the structure of **8** was determined to be glucozaluzanin C (Nagumo *et al.*, 1980).

Compounds (**1~8**) were tested for their cytotoxicity against human tumor cell lines (Table I). Compounds **1~4** and **8** showed non-specific significant cytotoxicity against five human tumor cell lines (0.36~5.54 μg/mL).

Table I. Cytotoxicity of compounds **1~8**

Compounds \Cancer Cell Lines	ED_{50} values [a]				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	2.64	1.32	2.50	2.72	1.05
2	0.39	1.21	1.24	0.47	0.35
3	2.81	1.52	5.54	2.80	1.48
4	2.72	1.38	0.36	1.49	1.42
5	13.56	10.07	5.14	12.60	19.31
6	18.54	21.13	13.22	18.62	15.49
7	>30.0	17.85	20.12	13.08	14.93
8	2.45	1.37	0.40	1.41	1.43
doxorubicin	0.018	0.071	0.009	0.008	0.381

a)ED_{50} was defined as the concentration (μg/mL) that caused a 50% inhibition of cell growth *in vitro*.

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REFERENCES

Ando, M., Kusaka, H., Ohara, H., Takase, K., Yamaoka, H. and Yanagi, Y., Studies on the syntheses of sesquiterpene lactones. *J. Org. Chem.*, 54, 1952-1960 (1989).

Banerji, A. and Pal, S., Constituents of *Piper sylvaticum*: structure of sylvatesmin. *J. Nat. Prod.*, 45, 672-675 (1982).

Bohlmann, F. and Gupta, R. K., Eremophilene and germacrene derivatives from *Senecio glanduloso-pilosus*. *Phytochemistry*, 21, 2595-2597 (1982).

Corrie, J. E. T., Green, G. H. and Taylor, W. C., The chemical constituents of Australian *Zanthoxylum* species. *Aust. J. Chem.*, 23, 133-145 (1970).

Deyama, T., Ikawa, T., Kitagawa, S. and Nishibe, S., The constituents of *Eucommia ulmoides* Oliv. V. Isolation of dihydroxydehydrodiconiferyl alcohol isomers and phenolic compounds. *Chem. Pharm. Bull*, 35, 1785-1789 (1987).

Gonzalez, A. G., Amaro, J., Fraga , B. M., and Luis, J., 3-Oxo-6β,-hydroxyolean-18-en-oic acid from *Orthopterygium huancuy*. *Phytochemistry*, 22, 1828-1830 (1983).

Hikino, H., Meguro, K., Kusano, G. and Takemoto, T., Structure of Mokko lactone. *Yakugaku Zasshi*, 87, 70-74 (1967).

Ishii, H., Ishkawa, T., Mihara, M. and Akaike, M., Studies on the chemical constituents of *Rutaceous* plants. XLVIII. The Chemical constituents of *Xanthoxylum ailanthoides* Sieb. et Zucc., *Yakugaku Zasshi*, 103, 279-292 (1983).

Jung, C. M., Kwon, H. C., Choi, S. Z., Lee, J. H., Lee, D. J., Ryu, S. N., and Lee, K. R., Phytochemical constituents of *Anisliaea acerifolia*. *Kor. J. Pharmacogn.*, 31, 125-129 (2000).

Kim, C. M., Shin, M. K., Lee, K. S. and Ahn, D. K., The Dictionary of Chinese Drugs, Jeong Dam Press, Seoul, pp. 727, 1360, 3448, 3870, 5476, 5721, 5918, (1999).

Kisiel, W., 8-Epidesacylcynaropicrin from Crepis capillaris. *Planta Med.*, 49, 246-247 (1983).

Kojima, H., Tominaga, H., Sato, S., and Ogura, H., Pentacyclic

triterpenoids from *Prunella vulgaris*. *Phytochemistry*, 26, 1107-1111 (1987).

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

Li, Y., and Jia, Z. J., Guaianolides from *Saussurea involucrate*. *Phytochemistry*, 28, 3395-3397 (1989).

Macrae, W. D. and Towers, G. H. N., Non-alkaloidal constituents of *Virola elongata* Bark. *Phytochemistry*, 24, 561-566 (1985).

Mahato, S. B., and Kundu, A. P., ^{13}C NMR spectra of pentacyclic triterpenoids - a complilation and some salient features. *Phytochemistry*, 37, 1517-1575 (1994).

Marco, J. A., Sanz, J. F., Albiach, R., Rustaiyan, A., and Habibi, Z., Bisabolene derivatives and sesquiterpene lactones from *Cousinia* species. *Phytochemistry*, 32, 395-400 (1993).

Marco, J. A., Sanz-Cervera, J. F., Yuste, A., and Oriola, M. C., Sesquiterpene lactones and dihydroflavonols from *Andryala* and *Urospermum* species. *Phytochemistry*, 36, 725-729 (1994).

Marco, J. A., Sanz-cervera, J. F., Garcia-lliso, V., Susanna, A., and Garcia-Jacas, N., Sesquiterpene lactones, lignans and aromatic esters from *Cheirolophus* species. *Phytochemistry*, 37, 1101-1107 (1994).

Nagumo, S., Izawa, K., Higashiyama, K. and Nagai, M., A bitter principle of *Pertya robusta* (Maxim.) Beauv.: Glucozaluzanin C. *Yakugaku Zasshi*, 100, 427-433 (1980).

Singhal, A. K., Chowdhury, P. K., and Sharma, R. P., Guaianolides from *Tricholepis glaberrima*. *Phytochemistry*, 21, 462-463 (1982).

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, H., Nakamura, T., Ichino, K. and Ito, K., A lignan from *Actinodaphne longifolia*. *Phytochemistry*, 28, 952-954 (1989).

Toshio, M., and Seigo, F., Sesquiterpene lactones from *Anisliaea acerifolia* Sch. Bip. and *A. dissecta* Franch. et Sav, *Chem. Pharm. Bull.*, 32, 3043-3046 (1984).

Yuuya, S., Hagiwara, H., Suzuki, T., Ando, M., Yamada, A., Suda, K., Kataoka, T. and Nagai, K., Guaianolides as immunomodulators. Synthesis and biological activities of dehydrocostus lactone, mokko lactone, eremanthin, and their derivatives. *J. Nat. Prod.*, 62, 22-30 (1999).

Zdero, C., Bohlmann, F., and Wasshausen, D. C., Guaianolides from *Brachylaena* species. *Phytochemistry*, 30, 3810-3811 (1991).