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Cytotoxic and COX-2 Inhibitory Constituents from the Aerial Parts of *Aralia cordata*

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Three diterpenes (**1**, **8**, and **9**), three triterpenes (**3**, **4**, and **7**), one saponin (**11**), four sterols (**2**, **5**, **6**, and **12**), and one cerebroside (**10**) were isolated from the EtOH extract of the aerial parts of *Aralia cordata* by repeated silica gel column chromatography. Their chemical structures were identified by comparing their physicochemical and spectral data with those published in literatures. All isolated compounds were evaluated for their cytotoxicity against L1210, K562, and LLC tumor cell lines using MTT assay. Of which, 3β,5α-dihydroxy-6β-methoxyergosta-7,22-diene (**6**) showed a potent cytotoxicity against all cell lines with IC_{50} values of 11.7, 11.9, and 15.1 μM, respectively, while compounds **1**, **5**, and **11** showed a moderate or weak cytotoxicity. These isolates were also examined for their inhibitory activity against COX-1 and COX-2. Although most compounds, except for **2**, **10**, and **12**, showed a strong inhibitory activity against COX-1, they exhibited a moderate or weak inhibitory activity against COX-2.

Key words: *Aralia cordata*, Diterpenes, Triterpenes, Sterols, Cerebroside, Cytotoxicity, COX-1, COX-2

INTRODUCTION

Aralia cordata Thunb. (Araliaceae) is a medicinal plant, which is distributed in Korea, China, and Japan. The root of *A. cordata* has been used for the treatment of rheumatism, lumbago, and lameness (Perry, 1980). On biological studies of principles from the root, a few diterpenes and essential oils have been elucidated as analgesic and anti-inflammatory agents (Han *et al.*, 1983; Okuyama *et al.*, 1991). Recently, eleven saponins, a number of terpens and polyacetylenes have been isolated and identified from the aerial parts of this plant (Yoshihara and Hirose, 1973; Ito *et al.*, 1978; Sawamura *et al.*, 1989). Among them, cytotoxicity of polyacetylenes, as well as antibacterial activity of farcarindiol, dehydrofalcarindiol, and *ent*-pimara-8(14),15-dien-19-oic acid have been reported (Park and Kim, 1995; Kwon and Lee, 2001). In our continuing study on this plant, twelve compounds, including three diterpenes, three triterpenes, one saponin, four sterols, and one cerebroside, were isolated and evaluated for their biological activity. The present paper reports the isolation, structural identification of these compounds and their cytotoxicity against L1210, K562, and LLC tumor cell lines, as well as their inhibitory activity against COX-1 and COX-2.

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MATERIALS AND METHODS

General experimental procedures

Melting points were determined on an Electrothemal apparatus. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. IR spectra and UV spectra were obtained on a JASCO 100 IR spectrophotometer and a JASCO V-550 UV/VIS spectrometer, respectively. FAB-MS was registered using a JEOL JMS-DX 300 spectrometer. ^{1}N-NMR and ^{13}C-NMR spectra were recorded with a Bruker DRX-300 spectrometer and chemical shift are expressed as δ values using TMS unit as an internal standard. Column chromatography was performed by using silica gel (Kieselgel 70-230 mesh and 230-400 mesh, Merck), and thin layer chromatography (TLC) was performed on pre-coated silica gel 60 F_{254} (0.25 mm, Merck).

Plant material

The aerial parts of *A. cordata* were collected in Daejeon, Korea, in November 2004, and identified by one of the authors (KiHwan Bae). A voucher specimen (CNU 1499) has been deposited in the herbarium at the College of Pharmacy, Chungnam National University, Daejeon, Korea.

Extraction and isolation

The dried aerial parts of *A. cordata* (4 kg) were extracted three times with EtOH at room temperature for 3 days, filtered and concentrated to yield an EtOH extract (300 g). The EtOH extract was suspended in H_2O and then partitioned successively with hexane, ethyl acetate (EtOAc), and butanol (BuOH) to yield a hexane-soluble fraction (85 g), an EtOAc-soluble fraction (63 g), and a BuOH-soluble fraction (82 g), respectively. The hexane fraction (85 g), exhibiting the most potent cytotoxicity against L1210 cells, was subjected to silica gel column chromatography eluted with gradient hexane-acetone (100:1 to 1:2) to afford four fractions (Fr. 1~4). Fr. 1 was further chromatographed on silica gel column using hexane-acetone (50:1) as an eluted solvent to afford compound **1** (1000 mg). Fr. 2 was rechromatographed on silica gel column using hexane-acetone (60:1 to 30:1) to yield compounds **2** (800 mg), **3** (10 mg), and **4** (20 mg), respectively. Repeated silica gel column chromatography of Frs. 3 and 4 using hexane-acetone (40:1 to 20:1) and hexane-acetone (20:1 to 4:1) gave compounds **5** (30 mg), **6** (30 mg), and **7** (25 mg). The EtOAc fraction (60 g), exhibiting also significant cytotoxicity against L1210 cells, was subjected to silica gel column chromatography eluted with gradient $CHCl_3$-MeOH (100:1 to 1:2) to afford five fractions (Fr. 5~9). Fr. 6 was chromatographed on silica gel column with gradient of hexane-acetone (20:1 to 10:1) to yield compounds **8** (20 mg) and **9** (130 mg). Fr. 7 was rechromatographed on silica gel column using hexane-acetone (10:1 to 5:1) to give compound **10** (60 mg). Repeated silica gel column chromatography of Fr. 9 using hexane-EtOAc (2:1 to 1:4) gave compounds **11** (15 mg) and **12** (50 mg).

Compound 1

Colorless needle; mp: 165-166°C; IR ν_{max} (KBr) cm^{-1}: 3400, 1690, 1460; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.66 (3H, s, H-20), 1.01 (3H, s, H-17), 1.23 (3H, s, H-18), 4.91 (1H, dd, J = 2.1, 11.7 Hz, H-16a), 4.96 (1H, dd, J = 1.8, 5.1 Hz, H-16b), 5.16 (1H, s, H-14), 5.71 (1H, dd, J = 10.5, 17.1 Hz, H-15); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 39.4 (C-1), 19.8 (C-2), 38.2 (C-3), 44.2 (C-4), 56.3 (C-5), 24.3 (C-6), 36.0 (C-7), 138.2 (C-8), 50.7 (C-9), 38.7 (C-10), 19.4 (C-11), 36.6 (C-12), 39.4 (C-13), 126.2 (C-14), 147.4 (C-15), 113.1 (C-16), 29.4 (C-17), 29.6 (C-18), 184.1 (C-19), 14.1 (C-20).

Compound 2

Colorless needle; mp: 158-160°C; IR ν_{max} (KBr) cm^{-1}: 3400, 1470; ^{1}H and ^{13}C-NMR were in accordance with authentic data (Hung and Yen, 2001).

Compound 3

Colorless needle; mp: 278-279°C; IR ν_{max} (KBr) cm^{-1}: 3580, 3050, 1635, 815; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.81, 0.91, and 0.95 (each 6H, s, 6×CH_3), 0.98 and 1.10 (each 3H, s, 2×CH_3), 3.17 (1H, m, H-3α), 5.53 (1H, dd, J = 4.0, 7.0 Hz, H-15); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 38.2 (C-1), 27.3 (C-2), 79.3 (C-3), 39.2 (C-4), 55.7 (C-5), 19.0 (C-6), 35.3 (C-7), 38.9 (C-8), 48.9 (C-9), 37.9 (C-10), 17.7 (C-11), 36.0 (C-12), 37.9 (C-13), 158.2 (C-14), 117.1 (C-15), 36.9 (C-16), 38.1 (C-17), 49.4 (C-18), 41.4 (C-19), 29.0 (C-20), 33.9 (C-21), 33.2 (C-22), 28.1 (C-23), 15.6 (C-24), 15.6 (C-25), 30.1 (C-26), 26.0 (C-27), 30.1 (C-28), 33.6 (C-29), 21.5 (C-30).

Compound 4

Colorless needle; mp: 269-271°C; IR ν_{max} (KBr) cm^{-1}: 3550, 3050, 1640, 815; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.80 (3H, s, H-24), 0.90 (3H, s, H-23), 0.92 (3H, s, H-29), 0.95 (3H, s, H-25), 0.97 (3H, s, H-30), 0.98 (3H, s, H-27), 1.08 (3H, s, H-26), 3.18 (1H, m, H-3á), 3.72 (2H, s, H-28), 5.45 (1H, dd, J = 4.0, 7.0 Hz, H-15); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 37.9 (C-1), 27.8 (C-2), 78.4 (C-3), 41.2 (C-4), 55.9 (C-5), 19.2 (C-6), 36.3 (C-7), 39.2 (C-8), 45.9 (C-9), 37.9 (C-10), 17.7 (C-11), 32.0 (C-12), 38.3 (C-13), 158.7 (C-14), 116.9 (C-15), 32.9 (C-16), 38.1 (C-17), 49.5 (C-18), 41.5 (C-19), 28.8 (C-20), 33.9 (C-21), 28.7 (C-22), 28.4 (C-23), 16.6 (C-24), 15.6 (C-25), 30.1 (C-26), 26.1 (C-27), 64.1 (C-28), 33.7 (C-29), 22.1 (C-30).

Compound 5

Colorless needle; mp: 180-182°C; IR ν_{max} (KBr) cm^{-1}: 3400, 1654, 1460; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.83 (3H, d, J = 5.0 Hz, H-27), 0.84 (3H, d, J = 5.0 Hz, H-26), 0.85 (3H, s, H-18), 0.91 (3H, d, J = 5.3 Hz, H-28), 0.98 (3H, d, J = 4.8 Hz, H-21), 1.08 (3H, s, H-19), 3.92 (1H, m, H-3), 5.12 (1H, dd, J = 8.0, 15.3 Hz, H-22), 5.20 (1H, dd, J = 7.5, 15.1 Hz, H-23), 6.25 (1H, d, J = 8.5 Hz, H-6), 6.49 (1H, d, J = 8.5 Hz, H-7); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 34.8 (C-1), 30.1 (C-2), 66.6 (C-3), 37.1 (C-4), 82.0 (C-5), 135.5 (C-6), 130.7 (C-7), 79.2 (C-8), 51.4 (C-9), 37.0 (C-10), 23.5 (C-11), 39.4 (C-12), 44.5 (C-13), 51.5 (C-14), 20.6 (C-15), 28.8 (C-16), 56.3 (C-17), 13.0 (H-18), 18.2 (C-19), 39.7 (C-20), 20.9 (C-21), 132.2 (C-22), 135.4 (C-23), 42.5 (C-24), 33.0 (C-25), 20.0 (C-26), 19.6 (C-27), 17.6 (C-28).

Compound 6

White powder; mp: 173-176°C; IR ν_{max} (KBr) cm^{-1}: 3400, 1640; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.58 (3H, s, H-18),

0.81 (3H, d, J = 7.0 Hz, H-26), 0.82 (3H, d, J = 7.0 Hz, H-27), 0.89 (3H, d, J = 6.5 Hz, H-28), 0.95 (3H, s, H-19), 0.97 (3H, d, J = 6.5 Hz, H-21), 3.20 (1H, d, J = 4.5 Hz, H-6), 3.40 (3H, s, OMe), 4.1 (1H, m, H-3), 5.15 (2H, dd, J = 8.0, 16.0 Hz, H-22), 5.25 (2H, dd, J = 7.5, 16.0 Hz, H-23), 5.40 (1H, m, H-7); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 32.2 (C-1), 30.6 (C-2), 68.0 (C-3), 39.6 (C-4), 78.0 (C-5), 82.5 (C-6), 114.5 (C-7), 144.2 (C-8), 44.5 (C-9), 38.1 (C-10), 22.3 (C-11), 39.6 (C-12), 44.5 (C-13), 55.5 (C-14), 23.3 (C-15), 27.8 (C-16), 56.3 (C-17), 14.2 (C-18), 18.6 (C-19), 40.7 (C-20), 20.2 (C-21), 132.0 (C-22), 135.5 (C-23), 43.2 (C-24), 33.2 (C-25), 20.1 (C-26), 21.8 (C-27), 18.3 (C-28), 58.3 (OMe).

Compound 7

White powder; mp: 196-198°C; IR ν_{max} (KBr) cm^{-1}: 3430, 1705; ^{1}H- and ^{13}C-NMR were in accordance with authentic data (Shim *et al.*, 2002).

Compound 8

White powder; mp: 275-277°C; IR ν_{max} (KBr) cm^{-1}: 3460, 1700; ^{1}H-NMR (300 MHz, CD_3OD): δ 0.98 (3H, s, H-20), 1.17 (3H, s, H-17), 1.33 (3H, s, H-18); ^{13}C-NMR (75 MHz, CD_3OD): δ 42.8 (C-1), 19.9 (C-2), 38.1 (C-3), 44.0 (C-4), 57.1 (C-5), 22.9 (C-6), 41.2 (C-7), 45.7 (C-8), 50.4 (C-9), 40.1 (C-10), 18.8 (C-11), 27.3 (C-12), 49.2 (C-13), 38.9 (C-14), 58.6 (C-15), 80.0 (C-16), 25.1 (C-17), 29.4 (C-18), 181.6 (C-19), 16.0 (C-20).

Compound 9

White powder; mp: 210-213°C; IR ν_{max} (KBr) cm^{-1}: 3450, 1695, 1460; FAB-MS *m/z* 337 $[M+H]^+$; ^{1}H-NMR (300 MHz, CD_3OD): δ 0.76 (3H, s, H-20), 0.90 (3H, s, H-17), 1.20 (3H, s, H-18), 3.40 (1H, dd, J = 8.7, 11.1 Hz, H-16a), 3.49 (1H, dd, J = 2.0, 11.1 Hz, H-15), 3.68 (1H, dd, J = 2.0, 8.7 Hz, H-16b), 5.36 (1H, s, H-14); ^{13}C-NMR (75 MHz, CD_3OD): δ 40.8 (C-1), 20.0 (C-2), 38.5 (C-3), 45.1 (C-4), 57.6 (C-5), 26.2 (C-6), 38.3 (C-7), 138.3 (C-8), 50.9 (C-9), 39.5 (C-10), 21.0 (C-11), 32.1 (C-12), 38.5 (C-13), 130.0 (C-14), 80.5 (C-15), 64.6 (C-16), 23.9 (C-17), 29.7 (C-18), 181.6 (C-19), 15.0 (C-20).

Compound 10

Amorphous white powder; mp: 214-216°C; IR ν_{max} (KBr) cm^{-1}: 3380, 1645, 1540, 1080, 1030, 720; ^{1}H-NMR (300 MHz, pyridine-d_5): δ 0.86 (6H, t-like, J = 6.8 Hz, 2×Me), 1.25 (s, $(CH_2)_n$), 3.85 (1H, m, H-5"), 3.98 (1H, t, J = 7.8 Hz, H-2"), 4.17 (2H, m, H-4, 3"), 4.27 (1H, m, H-3), 4.30 (1H, H-4"), 4.31 (1H, dd, J = 5.1, 12.9 Hz, H-6"), 4.46 (1H, dd, J = 3.1, 12.9 Hz, H-6"), 4.50 (1H, dd, J = 4.7, 10.5 Hz, H-1), 4.55 (1H, m, H-2'), 4.69 (1H, dd, J = 6.6, 10.5 Hz, H-1), 4.93 (1H, d, J = 7.9 Hz, H-1"), 5.26 (1H, m, H-2), 5.45 (1H, dt, J = 5.8, 15.6 Hz, H-8), 5.52 (1H, dt, J = 5.6, 15.6 Hz, H-9), 8.53 (1H, d, J = 9.1 Hz, N-H); ^{13}C-NMR (75 MHz, pyridine-d_5): δ 14.3 (Me), 23.0, 25.8, 26.7, 29.5, 29.6, 29.7, 29.8, 30.0,. 32.1 (C-7), 33.0 (C-10), 33.8, 35.6 (all CH_2), 51.7 (C-2), 62.6 (C-6"), 70.5 (C-1), 71.4 (C-4"), 72.4 (C-4, 2'), 75.1 (C-2"), 75.9 (C-3), 78.5 (C-3"), 78.6 (C-5"), 105.6 (C-1"), 130.5 (C-9), 130.8 (C-8), 175.6 (C-1').

Compound 11

Colorless needle; mp: 237-240°C; IR ν_{max} (KBr) cm^{-1}: 3440, 1735, 1075; ^{1}H-NMR (300 MHz, pyridine-d_5): δ 0.81, 0.89, 0.93, 1.02, 1.13, 1.22, and 1.24 (each 3H, s, 7×CH_3), 3.43 (1H, dd, J = 5.7, 9.7 Hz, H-3), 5.45 (1H, br s, H-12), 6.31 (1H, d, J = 7.7 Hz, H-1'); ^{13}C-NMR (75 MHz, pyridine-d_5): δ 78.4 (C-3), 176.5 (C-28), 96.1 (C-1'), 74.5 (C-2'), 79.7 (C-3'), 71.4 (C-4'), 79.3 (C-5'), 62.5 (C-6').

Compound 12

Whiter powder; mp: 285-287°C; IR ν_{max} (KBr) cm^{-1}: 3420, 1460, 1090; ^{1}H-NMR (300 MHz, pyridine-d_5): δ 0.66 (3H, s, H-18), 0.94 (3H, s, H-19), 5.36 (H, m, H-6); ^{13}C-NMR (75 MHz, pyridine-d_5): δ 37.9 (C-1), 30.4 (C-2), 78.7 (C-3), 40.2 (C-4),141.1 (C-5), 121.1 (C-6), 32.3 (C-7), 32.2 (C-9), 50.4 (C-9), 37.1 (C-10), 21.6 (C-11), 39.5 (C-12), 42.7 (C-13), 57.0 (C-14), 24.7 (C-15), 28.4 (C-16), 56.5 (C-17), 12.1 (C-18), 19.6 (C-19), 36.6 (C-20), 19.2 (C-21), 34.4 (C-22), 26.6 (C-23), 46.2 (C-24), 29.7 (C-25), 19.4 (C-26), 20.1 (C-27), 23.6 (C-28), 12.3 (C-29), 102.8 (C-1'), 75.5 (C-2'), 78.6 (C-3'), 71.9 (C-4'), 78.4 (C-5'), 63.3 (C-6').

Cytotoxicity assay

Cells were maintained in RPMI 1640 including L-glutamine (JBI) with 10% FBS (JBI) and 2% penicillin-streptomycin (GIBCO). Trypsin-EDTA was used to separate cell from the culture flask. All cell lines were cultured at 37°C in an atmosphere of 5% CO_2 incubator.

Cytotoxicity was measured by a modification of the Microculture Tetrazolium (MTT) assay (Mosmann, 1983). Viable cells were seeded in the growth medium (180 μL) into 96 well microtiter plate (1×10^4 cells per each well) and allowed to attach in 37°C, 5% CO_2 incubator. The test sample was dissolved in DMSO and adjusted to the final sample concentration raging from 1.875 μg/mL to 30 μg/mL by diluting with the growth medium. Each sample was prepared to triplicate. The final DMSO concentration was adjusted to < 0.1%. After standing 2 h, 20 μL of test sample were added to each wells in the same concentration of DMSO and were added in the control group. After 48 h test sample addition, 20 μL MTT (final concentration, 5 μg/mL) was added to the each well. Two hours later, the plate was centrifuged for 5 minutes in 1,500 rpm, the medium was removed and formed formazan crystals were dissolved with 150 μL DMSO. The optical density

(O.D.) was measured at 570 nm using a Titertek microplate reader (Multiskan MCC.340, Flow). The IC_{50} value was defined as the concentration of sample needed to reduce a 50% of absorbance relative to the vehicle-treated control.

Cyclooxygenase (COX) assay

The experiments were performed according to a slight modification of the method reported by Ylva (Ylva *et al.*, 1998). The enzyme (COX-1, 3.0 units, 0.43 μg protein; COX-2, 3.0 units, 0.39 μg protein) 10 μL was activated with 170 μL of a cofactor solution, which consisted of 1.3 mg/mL reduced glutathione (Sigma, U.S.A.), 1.3 mg/mL l-ephedrine (Sigma, U.S.A.) and 3 mg/mL reduced glutathione (Sigma, U.S.A.) in Tris-HCl buffer (pH 8.0) on ice for 4 min. An aliquot (10 μL) of the test solution (compound dissolved in DMSO) or the vehicle (DMSO) was added to the reaction tube and preincubated on ice for 10 min. After starting the reaction by adding 10 μL (0.02 ìCi) of 1-^{14}C arachidonic acid (50 μCi, NEC-661, NEN), the mixture was incubated for 20 min at 37°C. The reaction was quenched by adding 10 μL of 2N HCl. The produced prostaglandins and unmetabolized arachidonic acid were extracted with ethyl ether and separated by TLC (developing system, $CHCl_3$-MeOH-Acetic acid, 18:1:1). The authentic gross count of the ^{14}C-labelled PGE_2 was measured using electronic autoradiography. The inhibitory effects of the test samples were indicated by the amount of ^{14}C-labelled PGE_2 produced compared with that of the DMSO control (% control).

RESULTS AND DISCUSSION

As a result of measuring the inhibitory effects of the *A. cordata* extract and its solvent-fractions on the growth of L1210 cells, the hexane and EtOAc fractions exhibited significant cell growth inhibitions with 62.1 and 60.2%, respectively, at the concentration of 30 μg/mL (Table I). The subsequent bioassay-guided isolation of these two fractions resulted in the isolation of twelve compounds (**1**-**12**).

Compound **1** was obtained as a colorless needle with mp 165-166°C. The IR spectrum of **1** showed absorption bands at 3400-2500 and 1690 cm^{-1} (carboxylic group) and 1470 cm^{-1} (double bonds). The ^{1}H-NMR spectrum of **1** had resonances due to the presence of three methyl groups at δ 0.66, 1.01, and 1.23. In the ^{13}C-NMR spectrum, one carbonyl signal at δ 184.1, four olefinic signals at δ 113.1, 126.2, 138.2, and 147.4, indicating two double bonds, three methyl signals at δ 14.1, 29.4, and 29.6 were observed. Furthermore, the ^{13}C-NMR spectrum of **1** exhibited 20 carbon signals in total. These spectral data suggested that **1** was a pimarane-type diterpene. On the basis of the above evidences and comparison with the literature data (Sy and Brown, 1998), the structure of **1** was identified as *ent*-pimara-8(14),15-dien-19-oic acid.

Table I. Inhibitory effects of the *A. cordata* extract and its solvent-fractions on the growth of L1210 cells

Extract/fractions	Growth inhibition (%)[a]
	L1210
EtOH extract	35.4
Hexane fraction	62.1
EtOAc fraction	60.2
BuOH fraction	12.5

[a]All samples were tested at the concentration of 30 μg/mL

Compound **2** was easily deduced to be stigmasterol by direct comparison of spectral data with those reported in literature (Hung and Yen, 2001).

Compound **3** was obtained as a colorless needle with mp 278-279°C. It gave a positive Liebermann-Burchard test. The IR spectrum revealed the existence of hydroxyl absorbance band (3580 cm^{-1}). The ^{1}H-NMR spectrum of **3** showed eight tertiary methyl signals at δ 1.10, 0.98, 0.95, 0.91, and 0.81, an olefinic signal at δ 5.53 (1H, dd, J = 4.0, 7.0 Hz), and an oxygenated methine signal at δ 3.17 (1H, m). The ^{13}C-NMR spectrum of **3** revealed 30 carbon signals, which included eight methyls, ten methylenes, five methines, seven quaternary carbons. Moreover, the olefinic signals at δ 158.2 and 115.7 for C-14 and C-15 in the ^{13}C-NMR spectrum indicate a $\Delta^{14,15}$-taraxeren skeleton of compound **3**. On the basis of the above evidences and literature data (Sakurai *et al.*, 1987), the structure of **3** was identified as taraxerol.

Compound **4** also gave a positive Liebermann-Burchard test. The ^{1}H- and ^{13}C-NMR spectrum of **4** were closely similar to those of **3**, demonstrating that **3** and **4** had the same basic structure. The difference observed between them was that the C-28 signal of **4** was at δ 64.1, implying that it was an oxygenated methyl carbon, whereas the corresponding C-28 signal of **3** was at δ 30.1, which was a tertiary methyl carbon. Therefore, **4** was identified as myricadiol (Sakurai *et al.*, 1987).

Compound **5** was obtained as a colorless needle with mp 180-182°C. It was positive in peroxide reagent (Lee, 1991). The observation of six methyl signals in the ^{1}H-NMR spectrum of **5** at δ 0.83, 0.84, 0.85, 0.91, 0.98, and 1.08 indicated that the sterol fragment of **5** was an ergosterol derivative. The ^{13}C-NMR spectrum of **5** revealed 28 carbon signals, which included four olefinic signals at δ 135.5, 130.7, 132.2, and 135.4, an oxygenated carbon signal at δ 66.6, and two adjacent carbons to peroxy group at δ 82.0 and 79.2. All of these evidences were in good agreement with those of ergosterol endoperoxide (Kwon *et al.*, 2002).

Compound **6** was obtained as a white powder with mp 173-176°C. ^{1}H- and ^{13}C-NMR spectrum of **6** also indicated that it was an ergosterol derivative. ^{13}C-NMR spectrum of **6** supported the presence of 28 carbons, including four oxygen-bearing carbons, which appeared at δ 58.3, 68.0, 78.0, and 82.5, along with 4 olefinic carbons (δ 114.5, 144.2, 132.0, and 135.5). The ^{1}H-NMR spectrum also confirmed the presence of above functionalities, which appeared as two oxygenated proton signals at δ 3.20 (1H, d, *J* = 4.5 Hz) and 4.1 (1H, m), one methoxyl group signal at δ 3.40 (3H, s), three olefinic signals at δ 5.15 (2H, dd, *J* = 8.0, 16.0 Hz), 5.25 (2H, dd, *J* = 7.5, 16.0 Hz), and 5.4 (1H, m). The NOESY spectrum of **6** showed the correlations between H-3, H-6, and H-9, demonstrating that the methoxyl group at C-6 was β-orientation. Additional comparisons of the spectral data with published in literature (Kwon *et al.*, 2002) identified that **6** was 3β,5α-dihydroxy-6β-methoxyergosta-7,22-diene.

Compound **7** was obtained as a white powder with mp 196-198°C. It gave a positive Liebermann-Burchard test, suggesting a triterpenoid. Compound **7** was easily identified as oleanolic acid by comparison of their spectral

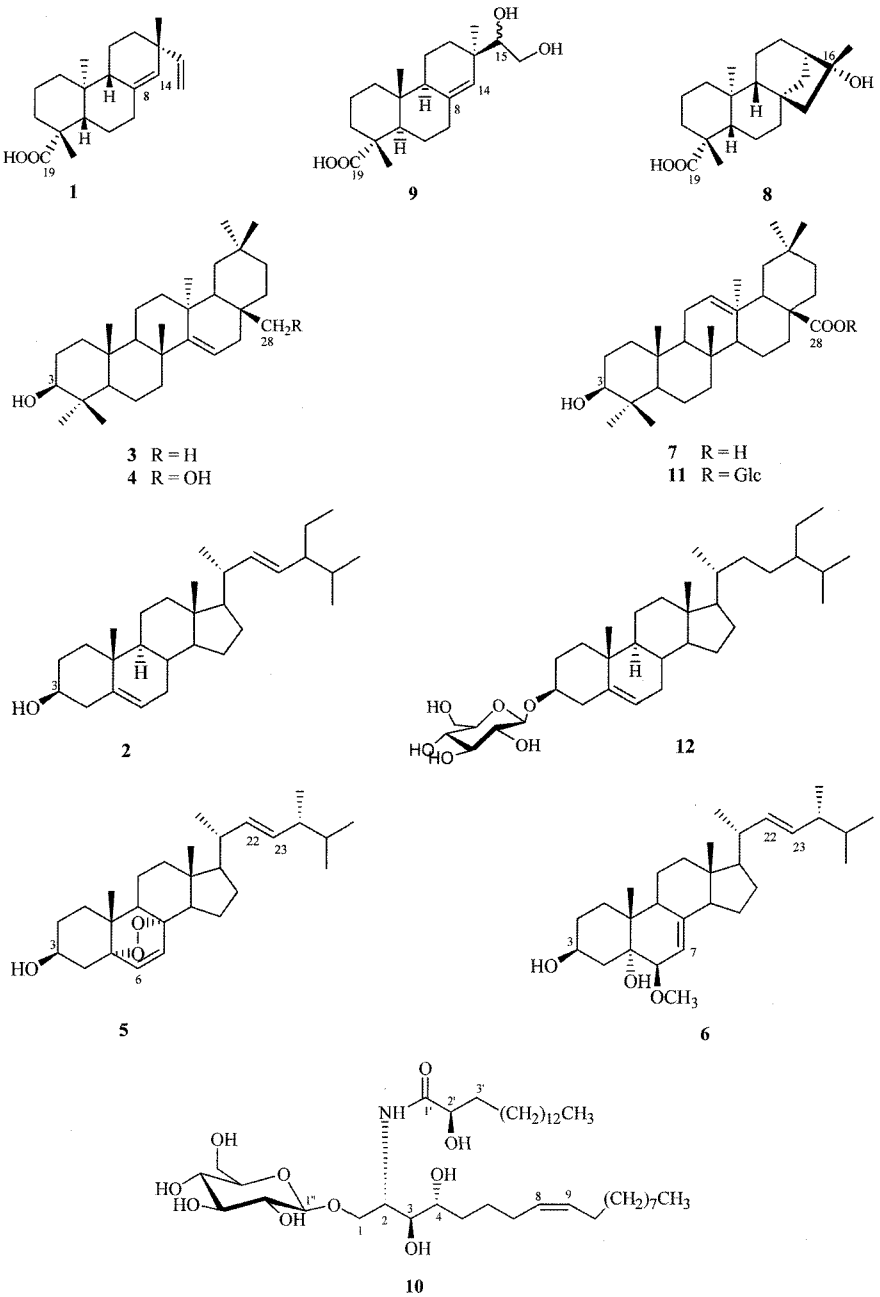

Fig. 1. Chemical structures of compounds **1-12** from *A. cordata*

properties (IR, ^{1}H-NMR, and ^{13}C-NMR) with those reported in literature (Shim *et al.*, 2002).

Compound **8** was obtained as a white powder with mp 275-277°C. The IR spectrum of **8** showed absorption bands at 3460-2600 and 1700 cm^{-1}, indicating the presence of a carboxylic group. Three singlets in the ^{1}H-NMR spectrum at δ 0.98, 1.17, and 1.33 suggested the presence of three methyl groups. Its ^{13}C-NMR and DEPT spectrum, which were in good agreement with a kauranoic acid structure, included three methyls, nine methylenes, two methines, one of which was oxygenated, and six quaternary carbons, one of which was carboxyl group of carboxylic acid corresponding to the signal at δ 181.6. Therefore, **8** was identified as 16α-hydroxy-*ent*-kauran-19-oic acid, which was accorded with those reported data (Cai *et al.*, 2003).

Compound **9** showed the molecular ion peak $[M+H]^+$ at *m/z* 337 in FAB-MS, suggesting the molecular formula $C_{20}H_{32}O_4$. The ^{13}C-NMR and DEPT spectrum displayed the presence of three methyls, eight methylenes, one of which was oxygenated, three methines, one of which was oxygenated, three quaternary carbons, two olefinic carbons, and a carboxylic group. The positive-ion FAB-MS of **9** showed a strong fragment ion peak at *m/z* 275, which was assignable to the loss of diol group (-$CHOHCH_2OH$) from the parent ion at m/z 337 $[M+H]^+$. In addition, the chemical shift value (δ_C 138.3) suggested a pimar-8(14)-ene skeleton (Cambie *et al.*, 1975). All these data suggested that **9** was a pimarene diterpenoid having a 1,2-dihydroxyethyl side chain and a double bond between C-8 and C-14. The placement of diol group was confirmed by analysis of the HMBC spectrum. In fact, HMBC cross-peaks between H-15 (δ_H 3.49) and C-12 (δ_C 32.1), C-13 (δ_C 38.5), C-14 (δ_C 130.0), C-16 (δ_C 64.6), and C-17 (δ_C 23.9) confirmed the placement of diol group at C-15. Base on the above evidences and comparison with the literature data (Sam *et al.*, 1991), **9** was identified as 15,16-dihyroxypimar-8(14)-en-19-oic acid.

The IR spectrum of compound **10** indicated the presence of hydroxyl groups (3380 cm^{-1}), amide functions (1645, 1540 cm^{-1}), glycosidic C-O (1080, 1030 cm^{-1}), and $(CH_2)_n$ functionalities (720 cm^{-1}). The NMR spectrum also showed typical of a sphingosine-type cerebroside possessing a sugar moiety (δ_H 4.93, 1H, d, J = 7.9 Hz, anomeric H; δ_C 105.6), an amide linkage (δ_H 8.53, 1H, d, J = 9.1 Hz, N-H; δ_C 175.6), and two long chain aliphatic moieties (δ_H 1.25, s, $(CH_2)_n$; δ_C 35.6). The fatty acid linked to C-2 of the sphingosine has been confirmed by the correlations between the NH proton and the carbon C-2. In addition, HMBC correlations of the carbonyl carbon at δ 175.6 with H-2' confirmed the presence of α-hydroxy fatty acid side chain. The large coupling constant of the double bond (15.6 Hz) indicated the *trans* (*E*) configuration. The chemical shifts at δ 70.5 (C-1), 51.7 (C-2), 75.9 (C-3), 72.4 (C-4), 175.6 (C-1'), and 72.4 (C-2') were identical with those of the data of other (2*S*,3*S*,4*R*)-phytosphingosine moieties (Sugiyama *et al.*, 1991). These results supported that the 1,3,4-trihydroxy phytosphingosine moiety of **10** possesses the 2*S*,3*S*,4*R* configuration. Therefore, **10** was identified as aralia cerebroside (Kang *et al.*, 1999).

^{1}H-NMR spectrum of **11** revealed seven methyl groups at δ 0.81, 0.89, 0.93, 1.02, 1.13, 1.22, and 1.24, one oxymethine proton at δ 3.43 (1H, dd, J = 5.7, 9.7 Hz), and two olefinic proton at δ 5.45 (1H, brs) in the aglycone moiety, which were in good agreement with those of oleanolic acid (Sati *et al.*, 1990). Moreover, anomeric proton of sugar moiety was shown at δ 6.31 (1H, d, J = 7.7 Hz). In the ^{13}C-NMR spectrum, sugar signals of **11** were identical to those of β-D-glycopyranoside (Sati *et al.*, 1990). Glycosidation shift was observed at C-28 of the aglycone, indicating that sugar moiety was attached at C-28. The accumulated evidence described above and comparison with the literature (Park *et al.*, 1999) identified that **11** was oleanolic acid 28-*O*-β-D-glycopyranosyl ester.

Compound **12** was obtained as a white powder, mp 285-287°C, gave a positive Liebermann-Burchard test. Acid hydrolysis of **12** revealed the presence of glucose as the sugar moiety and β-sitosterol as the aglycone. Additional detailed comparisons of the spectral data with published in literature (Jares *et al.*, 1990) identified that **12** was daucosterol.

Among the isolated compounds, compounds **5**, **6**, **9**, and **10** were isolated for the first time from this plant.

All isolated compounds were evaluated for their cytotoxicity against murine L1210 leukemia cells, human K562 cells, and mouse LLC Lewis lung carcinoma according to the MTT assay. As shown in Table II, compound **6**, 3β,5α-dihydroxy-6β-methoxyergosta-7,22-diene, exhibited the most potent cytotoxicity against all cell lines with IC_{50} values of 11.7, 11.9, and 15.1 μM, respectively, while compounds **1**, **5**, and **11** exhibited a moderate or weak cytotoxicity with IC_{50} values ranging from 28.2 to 98.2 μM. According to the previous data (Kwon *et al.*, 2002; Hirokazu *et al.*, 1988), compound **6** exhibited a potent cytotoxicity against various cancer cell lines such as A562 (non small cell lung adenocarcinoma), SK-OV-3 (ovarian), SK-MEL-2 (skin melanoma), XF498 (CNS), and HCT15 (colon). This potent cytotoxicity is because C-6 methoxyl group plays an important role for the appearance of cytotoxicity (Hirokazu *et al.*, 1988).

All isolated compounds were also examined for their inhibitory activity against COX-1 and COX-2. As a result presented (Table III), most of these compounds, except for **2**, **10**, and **12**, exhibited inhibitory activity against COX-1. Their inhibitory activities were stronger than positive control, aspirin (IC_{50}: 1804 μM). In the test for inhibitory

Table II. Cytotoxicity of compounds **1-12** from *A. cordata*

Compounds	IC_{50} (μM)[a]		
	L1210	K562	LLC
1	92.2	97.5	98.2
2	>100	>100	>100
3	>100	>100	>100
4	>100	>100	>100
5	28.2	43.9	58.3
6	11.7	11.9	15.1
7	>100	>100	>100
9	>100	>100	>100
10	>100	>100	>100
11	36.8	43.6	46.0
12	>100	>100	>100
AM[b]	1.5	2.8	3.5

[a]IC_{50} means the concentration of the sample required for 50% inhibition of cell growth

[b]Adriamycin was used as a positive control

Table III. Inhibitory activity against COX-1 and COX-2 of compounds **1-12** from *A. cordata*

Compounds	IC_{50} (μM)[a]	
	COX-1	COX-2
1	134.2	544.8
2	>1000	>1000
3	252.2	400.6
4	235.2	371.2
5	140.6	485.3
6	75.2	561.6
7	51.6	246.9
8	227.0	>1000
9	408.5	434.6
10	>1000	>1000
11	48.0	885.4
12	>1000	>1000
Aspirin[b]	1804	-
Indomethacin[b]	-	76.5

[a]IC_{50} means the concentration of the sample required for 50% inhibition of COX-1 or COX-2 activation

[b]Aspirin and indomethacin were used as positive controls

activity against COX-2, diterpene-type compounds (**1** and **9**), except for **8**, showed inhibitory activity with IC_{50} values of 544.8 and 434.6 μM, respectively. This result indicated that the pimarane compounds exhibited stronger inhibitory activity against COX-2 than the kaurane compounds (Dang *et al.*, 2005). Triterpene-type compounds (**3**, **4**, **7**, and **11**) also exhibited inhibitory activity. Of which, compound **7**, oleanolic acid, exhibited the most potent inhibitory activity against COX-2 with an IC_{50} value of 246.9 μM, while compound **11**, oleanolic acid 28-O-β-D-glycopyranosyl ester, which has a sugar moiety in the oleanolic acid exhibited a very weak inhibitory activity with an IC_{50} value of 885.4 μM. This suggested that the sugar moiety decreased the inhibitory activity against COX-2 of corresponding aglycone oleanolic acid.

In conclusion, sterols from *A. cordata*, especially ergosterol derivatives, exhibited a significant cytotoxicity *in vitro*, and terpenes from this plant, including diterpenes and triterpenes, exhibited a moderate or weak inhibitory activity against COX-2.

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