Arch Pharm Res Vol 28, No 1, 28-33, 2005

Archives of Pharmacal Research
http://apr.psk.or.kr

Inhibitory Constituents against Cyclooxygenases from *Aralia cordata* Thunb

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(Received October 25, 2004)

Seven diterpenes, four polyacetylenes, a lipid glycerol, and two sterols were isolated from the methylene chloride fraction of the root of *Aralia cordata*. Their chemical structures were determined as (-)-pimara-8(14),15-dien-19-oic acid (**2**), pimaric acid (**3**), (-)-kaur-16-en-19-oic acid (**4**), 17-hydroxy-*ent*-kaur-15-en-19-oic acid (**9**), 7α-hydroxy-(-)-pimara-8(14),15-dien-19-oic acid (**10**), 16α,17-dihydroxy-(-)-kauran-19-oic acid (**11**), 16-hydroxy-17-isovaleroyloxy-*ent*-kauran-19-oic acid (**12**), falcarindiol (**5**), dehydrofalcarindiol (**6**), dehydrofalcarindiol-8-acetate (**7**), falcarindiol-8-acetate (**8**), alpha-mono palmitin (**13**), stigmasterol (**1**), and daucosterol (**14**) by the spectral evidences. These compounds were tested with COX-1 and COX-2 inhibition assays. This study found that compounds **2**, **4**, **5**, **6**, **7**, **8**, and **10** inhibited COX-1 dependent conversion of the exogenous arachidonic acid to PGE_2 in a dose-dependent manner with IC_{50} values of 134.2 μM, 121.6 μM, 170 μM, 50.4 μM, 11.7 μM, 99.6 μM, and 69.6 μM, respectively. But, most of these compounds weakly inhibited COX-2 dependent PGE_2 generation. Among them, only compound **4** showed relatively significant inhibitory activity (IC_{50}: 127.6 μM).

Key words: *Aralia cordata*, Diterpenoids, Polyacetylenes, Lipid glycerol, Steroids, Anti-inflammatory, COX-1, COX-2

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used as analgesic or antipyretic for clinical treatment of anti-inflammatory disease such as arthritis, lumbago and rheumatism. It was well known that they act by inhibition of two isoforms COX-1 and COX-2, which are responsible for production of prostaglandins and thromboxanes from arachidonic acid (Emery *et al.*, 1999). Recently, several COX-2 selective inhibitors have been developed because these substances show less gastropathy effect than NSAIDs. Therefore, the search for these drugs is considered important.

The root of *Aralia cordata* Thunb. (Araliaceae) has been used as a traditional Chinese medicine for rheumatism, lumbago, and lameness (Kim *et al.*, 1998). From the root of this plant, a few numbers of diterpenes and essential oils have been identified. On biological studies of these compounds, diterpenes were known as active constituents for analgesic and anti-inflammatory agents (Han *et al.*, 1983; Okuyama *et al.*, 1991). Recent studies on aerial parts of the plant showed that falcarindiol, dehydrofalcarindiol and (-)-pimara-8(14),15-dien-19-oic acid are potent antibacterial constituents. Cytotoxic activity of polyacetylenes was also reported (Okuyama *et al.*, 1993; Kwon *et al.*, 2001). The present paper reports phytochemical constituents of the root of the plant and studies on anti-inflammatory activity using COX-1 and COX-2 assays.

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MATERIALS AND METHODS

General experiment 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. ^{1}H-NMR and ^{13}C-NMR spectra were recorded with a Bruker DRX-300 spectrometer. Column chromatography was performed on silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck).

Plant materials

The root of *Aralia cordata* was collected in Taejon, Korea, in March 2003 and identified by Professor KiHwan Bae and the voucher specimens (1499) were deposited at the College of Pharmacy, Chungnam University, Taejon, Korea.

Extraction and isolation

The dried root (3 kg) of *Aralia cordata* was extracted three times with hot MeOH under reflux. The resultant extracts were combined and concentrated under reduced pressure to afford 300 g of residue. The MeOH extract was suspended in water, and then partitioned successively with methylene chloride and concentrated. The methylene chloride fraction (80 g) was chromatographed on silica gel column. Gradient elution with $CHCl_3$-MeOH increasing proportion of MeOH gave 4 fractions.

Fraction 1 (26 g) was chromatographed on a silica gel column using stepwise gradient eluting with hexane-acetone (20:1) to afford compound **1** (20 mg), with hexane-acetone (10:1) to obtain compound **2** (2 g), **3** (5 mg), and **4** (150 mg).

Fraction 2 (14 g) was chromatographed on a silica gel column eluting with gradient of hexane-acetone (10:1→ 1:1). Fraction 2.1 was rechromatographed on a reversed phase silica gel column with MeOH-H_2O (4:1) to afford compound **5** (15 mg) and **6** (10 mg). Fraction 2.2 was repeatedly subjected to chromatography on a silica gel column eluting with $CHCl_3$-acetone (30:1) to give two fractions. Subfraction 2.2.1 was chromatographed on a reversed phase silica gel column eluting with MeOH-H_2O (3:1) to obtain compound **7** (15 mg) and **8** (10 mg). Compound **9** (10 mg) was recrystallized with hexane-acetone from fraction 2.3.

Fraction 3 (11 g) was chromatographed on a silica gel column with $CHCl_3$-MeOH (20:1). Subfraction 3.1 was chromatographed on a silica gel column with $CHCl_3$-acetone (10:1) to afford compound **10** (5 mg) and **11** (15 mg). The isolation of subfraction 3.2 on a reversed phase silica gel column with MeOH-H_2O (8:1) obtained compound **12** (40 mg).

Fraction 4 (10 g) was applied to a silica gel column chromatography using $CHCl_3$-MeOH (10:1) to yield compound **13** (25 mg). Subfraction 4.1 was subjected to an YMC silica gel column chromatography eluted with MeOH-H_2O-acetic acid (70:15:0.8) to afford compound **14** (20 mg).

Stigmasterol (1)

IR ν_{max} cm^{-1}: 3410 (OH), 1460 (C=C); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 5.35 (1H, d, *J* = 5.4 Hz, H-6), 3.53 (1H, m, H-3), 1.01 (3H, s, H-19), 0.67 (3H, s, H-18).

(-)-Pimara-8(14),15-dien-19-oic acid (2)

Colorless needle (hexane); mp: 165-166°C; $[\alpha]_D$-120° (c= 0.75, $CHCl_3$); UV λ_{max} MeOH nm (log ε): 213 (3.34); IR ν_{max} cm^{-1}: 1690 (C=O); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 5.69 (1H, dd, *J* = 10.5, 17.1 Hz, H-15), 5.17 (1H, s, H-14), 4.96 (1H, dd, *J* = 2.1, 5.1 Hz, H-16b), 4.90 (1H, dd, *J* = 2.1, 10.5 Hz, H-16a), 1.32 (3H, s, H-18), 1.02 (3H, s, H-17), 0.68 (3H, s, H-20); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 39.6 (C-1), 20.0 (C-2), 38.4 (C-3), 44.4 (C-4), 56.5 (C-5), 24.5 (C-6), 36.2 (C-7), 138.3 (C-8), 50.9 (C-9), 38.9 (C-10), 19.6 (C-11), 36.8 (C-12), 39.6 (C-13), 128.4 (C-14), 147.6 (C-15), 113.2 (C-16), 29.7 (C-17), 14.1 (C-18), 183.9 (C-19), 29.5 (C-20).

Pimaric acid (3)

Needle; mp: 206-207°C; UV λ_{max} MeOH nm (log ε): 208 (3.09); IR ν_{max} cm^{-1}: 1690 (C=O); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 5.70 (1H, dd, *J* = 10.5, 17.1 Hz, H-15), 5.17 (1H, d, *J* = 0.9 Hz, H-14), 4.95 (1H, m, H-16b), 4.90 (1H. dd, *J* = 2.1, 15.3 Hz, H-16a), 1.23 (3H, s, H-18), 0.99 (3H, s, H-17), 0.80 (3H, s, H-20); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 39.6 (C-1), 25.2 (C-2), 37.5 (C-3), 47.6 (C-4), 49.2 (C-5), 25.2 (C-6), 35.7 (C-7), 138.3 (C-8), 51.8 (C-9), 38.2 (C-10), 19.3 (C-11), 36.0 (C-12), 38.9 (C-13), 128.8 (C-14), 147.7 (C-15), 113.2 (C-16), 29.8 (C-17), 15.4 (C-18), 185.0 (C-19), 17.2 (C-20).

(-)-Kaur-16-en-19-oic acid (4)

Cubic crystal (hexane-acetone); mp: 178-180°C; $[\alpha]_D$-110° (c=1.00, $CHCl_3$); UV λ_{max} MeOH nm (log ε): 211 (3.23); IR ν_{max} cm^{-1}: 3450 (OH), 1690 (C=O), 1470 (C=C); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 4.76-4.82 (1H each, br s, H-17), 2.66 (1H, br s, H-13), 2.07 (2H, d, *J* = 3.0 Hz, H-6), 1.61-1.63 (2H, m, H-11), 1.4-1.6 (4H, m, H-7, 12), 1.26 (3H, s, H-18), 1.0-2.2 (4H, m, H-2, 3), 1.07-1.09 (1H, m, H-5), 0.98 (3H, s, H-20), 0.83 (2H, dd, *J* = 4.2, 13.2 Hz, H-1); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 41.1 (C-1), 19.5 (C-2), 38.2 (C-3), 44.3 (C-4), 57.5 (C-5), 22.2 (C-6), 41.7 (C-7), 44.6 (C-8), 55.5 (C-9), 40.7 (C-10), 18.8 (C-11), 33.5 (C-12), 44.1 (C-13), 40.1 (C-14), 49.4 (C-15), 156.3 (C-16), 103.4 (C-17), 29.4 (C-18), 184.7 (C-19), 16.0 (C-20).

Falcarindiol (5)

Oil; UV λ_{max} MeOH nm (log ε): 234 (4.01), 243 (3.26), 257 (2.64), 271 (2.35), 284 (1.95); IR ν_{max} cm^{-1}: 3350 (OH), 2230, 2150 (C=C), 1460 (C=C); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 5.93 (1H, dd, *J* = 2.1, 9.9 Hz, H-2), 5.51 (1H, m, H-10), 5.47 (1H, dt, *J* = 2.1, 11.1 Hz, H-1a), 5.25 (1H, dt, *J* = 2.1,

10.2 Hz, H-1b), 5.21 (1H, d, J = 8.1 Hz, H-8), 4.95 (1H, br d, J = 5.4 Hz, H-3), 3.30 (4H, m, H13, 16), 2.10 (2H, m, H-11), 1.40 (2H, m, H-12), 0.89 (3H, t, J = 6.3, 6.9 Hz, H-17); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 117.7 (C-1), 136.2 (C-2), 63.9 (C-3), 80.3 (C-4), 70.7 (C-5), 69.1 (C-6), 78.7 (C-7), 59.1 (C-8), 128.1 (C-9), 135.1 (C-10), 32.2 (C-11), 29.7 (C-12), 29.5 (C-13), 29.5 (C-14), 28.1 (C-15), 23.0 (C-16), 14.5 (C-17).

Dehydrofalcarindiol (6)

Oil; UV λ_{max} MeOH nm (log ε): 226 (3.83), 245 (2.34), 259 (1.93), 269 (1.57); IR ν_{max} cm^{-1}: 3350 (OH), 2230, 2150 (C=C), 1640, 1420 (C=C); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 5.91 (1H, dddd, J = 5.1, 5.4, 9.9, 10.2 Hz, H-2), 5.76 (2H, dddd, J = 3.3, 10.2, 16.8, 17.1 Hz, H-16), 5.56 (1H, m, H-10), 5.29 (1H, ddd, J = 0.9, 1.5, 10.2 Hz, H-1b), 5.26 (1H, ddd, J = 0.9, 1.2, 10.2 Hz, H-1a), 5.21 (1H, d, J = 8.1 Hz, H-8), 5.04 (1H, dd, J = 1.5, 3.6 Hz, H-17b), 4.94 (1H, m, H-17a), 2.03 (2H, m, H-11, 15), 1.35 (4H, m, H-12, 14); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 117.2 (C-1), 139.4 (C-2), 63.9 (C-3), 78.7 (C-4), 70.7 (C-5), 69.1 (C-6), 80.2 (C-7), 59.0 (C-8), 128.2 (C-9), 134.9 (C-10), 28.3 (C-11), 29.5 (C-12), 29.1 (C-13), 29.0 (C-14), 34.1 (C-15), 139.4 (C-16), 114.7 (C-17).

Dehydrofalcarindiol-8-acetate (7)

Oil; UV λ_{max} MeOH nm (log ε): 212 (3.29), 269 (0.71), 285 (0.58); IR ν_{max} cm^{-1}: 3450 (OH), 1750 (C=O), 1230 (C-O); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 5.95 (1H, dd, J = 5.4, 10.2 Hz, H-3), 5.89 (1H, dd, J = 5.4, 10.2 Hz, H-2), 5.78 (1H, dddd, J = 3.3, 3.6, 10.5, 23.7 Hz, H-16), 2.11 (3H, s, H-19), 1.22 (6H, m, H-12, 13, 14); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 117.7 (C-1), 136.1 (C-2), 63.8 (C-3), 76.9 (C-4), 70.5 (C-5), 69.6 (C-6), 78.9 (C-7), 60.5 (C-8), 124.3 (C-9), 136.7 (C-10), 28.2 (C-11), 29.3 (C-12), 29.1 (C-13), 29.0 (C-14), 34.1 (C-15), 139.4 (C-16), 114.7 (C-17), 169.9 (C-18), 21.3 (C-19).

Falcarindiol-8-acetate (8)

Oil; IR ν_{max} cm^{-1}: 3310 (OH), 1750 (C=O), 1230 (C-O); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 5.95 (1H, dd, J = 5.4, 10.2 Hz, H-3), 5.89 (1H, dd, J = 5.4, 10.2 Hz, H-2), 5.64 (1H, ddd, J = 0.6, 0.9, 1.2 Hz, H-10), 5.45 (1H, m, H-9), 4.94 (1H, d, J = 5.1 Hz, H-8), 2.12 (4H, m, H-11, 15), 2.09 (3H, s, H-19), 1.29 (6H, m, H-12, 13, 14), 0.88 (3H, t, J = 6.3, 6.9 Hz, H-17); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 117.7 (C-1), 136.2 (C-2), 63.8 (C-3), 76.9 (C-4), 70.5 (C-5), 69.6 (C-6), 78.9 (C-7), 60.5 (C-8), 124.2 (C-9), 136.8 (C-10), 28.2 (C-11), 29.7 (C-12), 29.1 (C-13), 29.5 (C-14), 32.17 (C-15), 22.3 (C-16), 14.5 (C-17), 169.8 (C-18), 21.3 (C-19).

17-Hydroxy-*ent*-kaur-15-en-19-oic acid (9)

Colorless needle (hexane-acetone); mp: 129-131°C; UV λ_{max} MeOH nm (log ε): 202 (3.52); IR ν_{max} cm^{-1}: 3400 (OH), 1470 (C=C); ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 5.61 (1H, s, H-15), 1.35 (3H, s, H-20), 1.19 (3H, s, H-18); ^{13}C-NMR (75 MHz, pyridine-d_5) δ: 41.3 (C-1), 20.0 (C-2), 44.4 (C-3), 40.3 (C-4), 48.3 (C-5), 21.8 (C-6), 38.9 (C-7), 49.3 (C-8), 57.0 (C-9), 44.1 (C-10), 19.4 (C-11), 26.1 (C-12), 41.7 (C-13), 40.0 (C-14), 135.1 (C-15), 148.2 (C-16), 60.7 (C-17), 16.0 (C-18), 180.2 (C-19), 29.5 (C-20).

7α-Hydroxy-(-)-pimara-8(14),15-dien-19-oic acid (10)

Needle; mp: 286-288°C (uncorrected); IR ν_{max} cm^{-1}: 3350 (OH), 1695 (C=O), 1470 (C=C); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 6.22 (1H, s, H-14), 5.76 (1H, dd, J = 10.2, 17.4 Hz, H-15), 4.93 (2H, m, H-16), 4.31 (1H, dd, J = 4.2, 11.7 Hz, H-7), 2.77 (1H, dd, J = 2.4, 5.4 Hz, H-6b), 2.54 (1H, q, J = 12.6 Hz, H-6a), 2.16 (2H, dt, J = 3.3, 10.2 Hz, H-2), 1.73 (2H, m, H-1), 1.42-1.63 (2H, m, H-11), 1.40 (3H, s, H-17), 1.15-1.63 (1H, m, H-5), 1.09 (3H, s, H-20), 1.02 (2H, m, H-3), 0.98 (3H, s, H-18); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 39.8 (C-1), 20.51 (C-2), 38.9 (C-3), 44.2 (C-4), 53.5 (C-5), 35.4 (C-6), 72.8 (C-7), 142.0 (C-8), 49.2 (C-9), 39.7 (C-10), 19.9 (C-11), 36.1 (C-12), 38.7 (C-13), 125.3 (C-14), 147.8 (C-15), 113.2 (C-16), 29.8 (C-17), 14.4 (C-18), 29.6 (C-19), 180.0 (C-20).

16α,17-Dihydroxy-(-)-kauran-19-oic acid (11)

White powder; mp: 254-256 °C; IR ν_{max} cm^{-1}: 3400 (OH), 1695 (C=O), 1470 (C=C), 1220 (C-O); ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 4.13 (1H, d, J = 8.1 Hz, H-17a), 4.04 (1H, d, J = 8.1 Hz, H-17b), 1.36 (3H, s, H-18), 1.20 (3H, s, H-20); ^{13}C-NMR (75 MHz, pyridine-d_5) δ: 41.2 (C-1), 20.0 (C-2), 38.8 (C-3), 44.0 (C-4), 57.1 (C-5), 23.1 (C-6), 42.9 (C-7), 45.1 (C-8), 56.4 (C-9), 40.2 (C-10), 19.1 (C-11), 26.9 (C-12), 46.0 (C-13), 37.9 (C-14), 44.1 (C-15), 81.7 (C-16), 65.6 (C-17), 29.5 (C-18), 180.1 (C-19), 16.2 (C-20).

16-Hydroxy-17-isovaleroyloxy-*ent*-kauran-19-oic acid (12)

Needle; mp: 182-184°C; IR ν_{max} cm^{-1}: 3450 (OH), 1740 (C=O), 1450 (C=C); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 4.25 (2H, d, J = 3 Hz, H-17), 1.25 (3H, s, H-18), 1.00 (6H, d, J = 4.5 Hz, H-4α, 5α), 0.97 (3H, s, H-20); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 40.9 (C-1), 19.4 (C-2), 38.2 (C-3), 44.0 (C-4), 57.2 (C-5), 22.4 (C-6), 42.2 (C-7), 45.2 (C-8), 56.1 (C-9), 40.1 (C-10), 18.8 (C-11), 26.6 (C-12), 46.9 (C-13), 37.5 (C-14), 53.3 (C-15), 80.5 (C-16), 68.3 (C-17), 29.3 (C-18), 183.2 (C-19), 15.9 (C-20), 173.6 (C-1α), 43.8 (C-2α), 26.2 (C-3α), 22.8 (C-4α), 22.8 (C-5α).

Alpha-mono palmitin (13)

Colorless crystal; mp: 74-76°C; FAB-MS (positive) *m/z* 331 $[M+H]^+$, 353 $[M+Na]^+$; IR ν_{max} cm^{-1}: 3300 (OH), 1730 (C=O), 1470 (C=C); 1220, 1120 (C-O); ^{1}H-NMR (300 MHz,

$CDCl_3$) δ: 4.14 (2H, dddd, J = 4.5, 6.0, 11.4, 11.7 Hz, H-1), 3.92 (1H, m, H-2), 3.74 (2H, dddd, J = 4.5, 5.7, 11.4 Hz, H-3), 2.34 (2H, t, J = 7.5 Hz, H-2), 1.60 (2H, m, H-3), 1.28 (24H, m, H-4~15), 0.88 (3H, t, J = 6.6 Hz, H16); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 174.7 (C- 1), 34.5 (C-2), 25.3 (C-3), 29.5-30.1 (C-4~13), 32.3 (C-14), 23.0 (C-15), 14.4 (C-16), 63.7 (C-1), 70.7 (C-2), 65.6 (C-3).

Daucosterol (14)

White powder; mp: 285-287 °C; IR ν_{max} cm^{-1}: 3420 (OH), 1460 (C=C), 1090 (C-O); ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 0.93 (3H, s, H-19), 0.65 (3H, s, H-18); ^{13}C-NMR (75 MHz, pyridine-d_5) δ: 37.7 (C-1), 30.5 (C-2), 78.8 (C-3), 40.2 (C-4), 141.1 (C-5), 122.1 (C-6), 32.3 (C-7), 32.2 (C-8), 50.6 (C-9), 37.1 (C-10), 21.5 (C-11), 39.5 (C-12), 42.7 (C-13), 57.0 (C-14), 24.7 (C-15), 28.7 (C-16), 56.5 (C-17), 12.2 (C-18), 19.6 (C-19), 36.6 (C-20), 19.2 (C-21), 34.4 (C-22), 26.6 (C-23), 46.3 (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), 78.3 (C-5), 63.1 (C-6).

Effect of various compounds on the COX-1 or COX-2 enzyme activity

COX-1, COX-2, [1-^{14}C] arachidonic, and other reagents were purchased from Sigma Chemical Company (St. Louis, MO, USA). Test compounds dissolved in DMSO were added to each assay buffer. After 10 minutes of pre-incubation with COX-1 or COX-2 (3units), [1-^{14}C] arachidonic acid was added and incubated for 20 minutes at 37°C. Reaction was terminated by addition of 2 M HCl and ethyl ether to separate formed-prostaglandins. Ethyl ether solution was removed at 37°C and the remained was dissolved in acetone and chromatographed on TLC. The prostaglandin products were quantified with electronic autoradiography.

RESULTS AND DISCUSSION

The compound **1** was obtained as a colorless needle from fraction 1 and identified as stigmasterol by a comparison with the ^{1}H-NMR, ^{13}C-NMR literature data (Hung *et al.*, 2001). Compounds **2** and **3** were isolated from fraction 1 as crystals. Their IR spectrums showed absorptions at 1690, 1690 cm^{-1} (carbonyl group) and 1460, 1450 cm^{-1} (C=C), respectively. The ^{1}H-NMR spectrum of these compounds had resonances due to the presence of three methyl groups at δ 0.68, 1.02, and 1.32 (compound **2**), δ 0.80, 0.99, and 1.23 (compound **3**). The ^{13}C-NMR spectrum of **3** was in good agreement with those of compound **2** except for the downfield shift of signal at δ 17.2 (C-20). The difference implied that **2** and **3** were the same pimarane skeleton but different of configuration at C-20 methyl group. On the basis of the above evidences and comparisons with the literature data (Shibata *et al.*, 1967; Masuto *et al.*, 1976; Rahman *et al.*, 1992), the structures of **2** and **3** were identified as (-)-pimara-8(14),15-dien-19-oic acid and pimaric acid, respectively. Compound **4** was identified as (-)-kaur-16-en-19-oic acid by comparisons with the authentic data (Tanaka *et al.*, 1972; Okuyama *et al.*, 1991).

Compounds **5, 6, 7,** and **8** were suggested as polyacetylenes by comparisons with the published values and they were assigned as falcarindiol (Zheng *et al.*, 1999; Andreas *et al.*, 2003), dehydrofalcarindiol (Bernart *et al.*, 1996), dehydrofalcarindiol-8-acetate, falcarindiol-8-acetate (Park *et al.*, 1995), respectively.

The compound **9** was obtained as a colorless needle. Its IR spectrum had absorption typical of a hydroxyl group (3400 cm^{-1}). The ^{1}H-NMR spectrum showed two tertiary methyl groups at δ 1.19 and 1.35, a methine group at δ 5.61 (1H, s, H-15). The ^{13}C-NMR spectrum revealed the presence of 20 carbons. Base on the reported literature data, compound **9** was identified as 17-hydroxy-*ent*-kaur-15-en-19-oic acid (Yahara *et al.*, 1974).

The compound **10** exhibited the similar of the spectrum to compound **2**, but a major difference was an absence of one hydroxyl group at δ 4.31 (dd, J = 4.2, 11.7 Hz). The ^{13}C-NMR spectrum indicated that one hydroxyl group was placed at C-7 due to high chemical shift value δ 72.84. The above evidences led to identification of 7α-hydroxy-(-)-pimara-8(14),15-dien-19-oic acid (Mishashi *et al.*, 1969). This is the first reported on ^{13}C-NMR data of upon compound.

The compound **11** was identified as 16α,17-dihydroxy-(-)-kauran-19-oic acid by a direct comparison of IR, ^{1}H-NMR, and ^{13}C-NMR spectral data with those of published literature (Silva *et al.*, 1999). The compound was reported to have activity against HIV reverse transcriptase at a concentration 33 mg/mL (Chang *et al.*, 1998).

The compound **12** was isolated from fraction 3 as a needle. Its IR spectrum showed absorptions at 3450 cm^{-1}, which was assigned to hydroxyl stretching mode, and 1740 cm^{-1}, which was typical to carbonyl group. The ^{1}H-NMR had resonances due to the presences of two tertiary methyl groups at δ 0.97 and 1.25, two secondary methyl groups at δ 1.00 (d, J = 4.5 Hz). The ^{13}C-NMR spectrum of **12** revealed 25 carbons including 6 quaternary, 4 methine, 11 methylene, and 4 methyl carbons. The chemical shifts in the ^{1}H-NMR and ^{13}C-NMR spectra of 13 were compared with those of compound **11** which gave similar values except those of C-17 and those of isovaleroyloxy group. Structure of **12** was determined as 16-hydroxy-17-isovaleroyloxy-*ent*-kauran-19-oic acid by a comparison with the literature data (Zhang *et al.*, 1999). To our best knowledge, this compound was the first time isolated from the plant.

The positive FAB-MS of compound **13** showed ion peaks at *m/z* 331 $[M+H]^+$ and 353 $[M+Na]^+$ corresponding to the molecular formula $C_{19}H_{38}O_4$. The IR spectrum had absorptions at 3300 cm^{-1} (hydroxyl group), 1730 cm^{-1} (carbonyl group) and 1120 cm^{-1} (C-O-C). On the basis of ^{1}H-NMR, ^{13}C-NMR and a comparison with the authentic data, compound **13** was assigned as alpha-mono palmitin.

Compound **14** was obtained as a white powder and identified as daucosterol by a comparison of ^{1}H-NMR and ^{13}C-NMR with the literature data (Sang *et al.*, 2002). The compounds **13** and **14** were isolated for the first time from this plant.

Inhibitory activity against COX-1 and COX-2

All fractions from the methylene chloride soluble fraction of MeOH extract were evaluated for inhibitory activity against COX-1 and COX-2. The method was a slight modification of that described by Ylva *et al.* (1998). The fractions 1, 2, and 3 showed significant COX-1 inhibition activities. Repeated chromatography of the active fractions resulted in the isolation of 14 compounds. All compounds in Table I showed a stronger inhibitory activity against COX-1 than aspirin (IC_{50} of 1.804 mM). Diterpene-type compounds from *Aralia cordata* showed difference in inhibition values. Generally, the pimarane compounds exhibited a stronger inhibitory activity against COX-1 than the kaurane compounds. Compound **10**, which has a hydroxyl group at the C-7, showed a higher activity than compounds **2** and **3**. Noteworthy, compound **4**, possessing a double bond at C-16, also presented a high inhibition effect against COX-1 (IC_{50} value of 121.6 μM). The other kaurane compounds did not exhibit inhibitory activity. This result suggested that the double bond at C-16 position may be attributed to COX-1 inhibition effect. Polyacetylenes **5**, **6**, **7**, and **8** showed considerable activities against COX-1 with IC_{50} of 170.3 μM, 50.4 μM, 99.6 μM, and 11.7 μM, respectively. However, compounds **2**, **3**, **4**, **6**, and **7** exhibited lower COX-2 inhibition activities than the positive control (indomethacine, IC_{50} of 76.5 μM) and the others are non-acitve. Among them, compound **4**

Table I. Inhibitory activities against COX-1 and COX-2 of the compounds isolated from *Aralia cordata*

Compounds[a]	COX-1 (IC_{50}, μM)[b]	COX-2 (IC_{50}, μM)[b]
2	134.2	600.4
3	331.6	592.1
4	121.6	127.6
5	170.3	NA[c]
6	50.4	460.1
7	11.7	432.2
8	99.6	NA
10	69.6	NA
Aspirin[d]	1804	
Indomethacin[d]		76.5

[a] Compounds non-active with both COX-1 and COX-2 were not showed in the Table. [b] Results are mean of triplicate. [c] Non-active. [d] Aspirin and indomethacin were used as the positive controls.

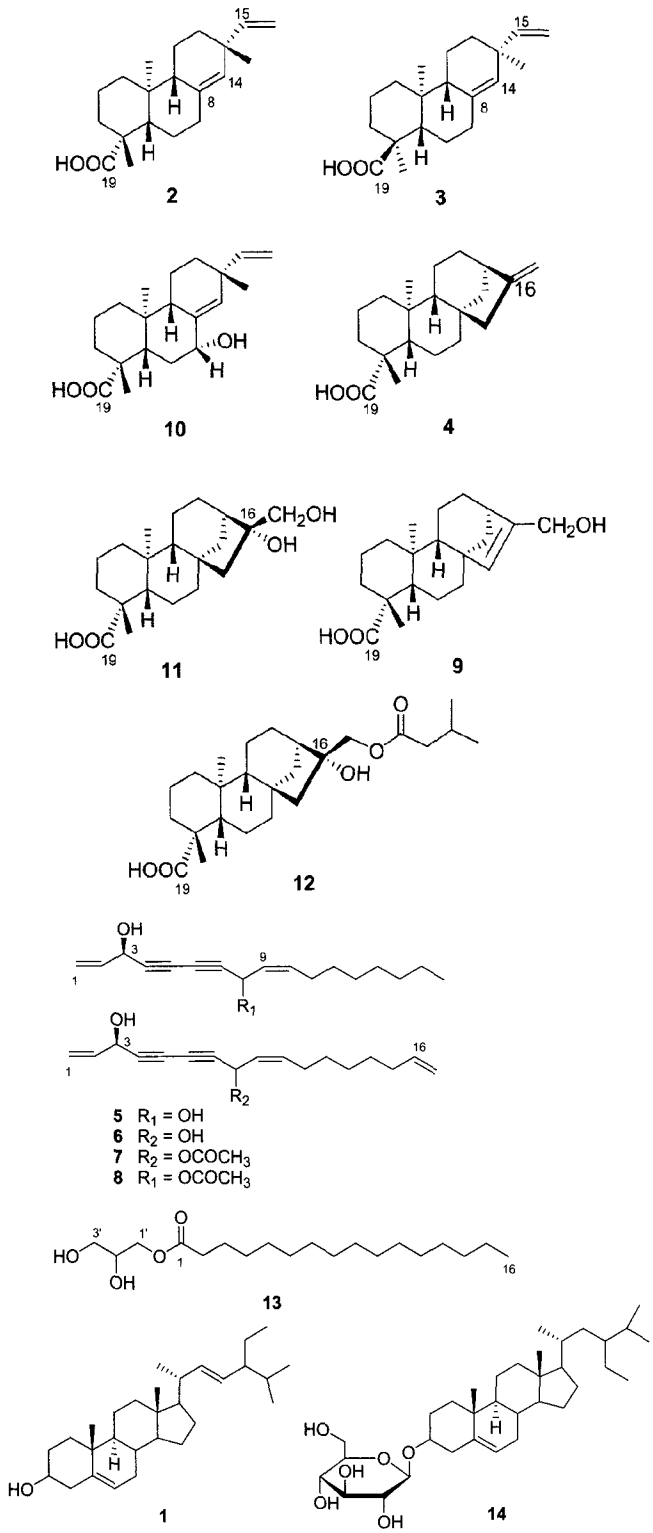

Fig. 1. The structures of compounds isolated from *Aralia cordata*

showed the most effective activity against COX-2 with IC_{50} of 127.6 μM.

ACKNOWLEDGEMENT

This work was supported by the grant (PF0320302-00) from the Plant Diversity Research Center of the 21st century Frontier Program funded by the Ministry of Science and Technology of Korea.

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