Arch Pharm Res Vol 28, No 10, 1135-1141, 2005

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

Inhibitory Effect on TNF-α-Induced IL-8 Production in the HT29 Cell of Constituents from the Leaf and Stem of *Weigela subsessilis*

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(Received January 18, 2005)

Twelve compounds were isolated from the MeOH extract of the leaf and stem of the Korean endemic plant *Weigela subsessilis* L. H. Bailey. Their chemical structures were elucidated on the basis of physicochemical and spectroscopic data and by comparison with those of published literatures. These compounds were identified as three sterols, β-sitosterol acetate (**2**), β-sitosterol (**3**), daucosterol (**11**), eight triterpenoids, squalene (**1**), ursolic acid (**4**), ilekudinol A (**5**), corosolic acid (**6**), ilekudinol B (**7**), esculentic acid (**8**), pomolic acid (**9**), asiatic acid (**10**), and one iridoid glycoside, alboside I (**12**). This is the first report pertaining to the isolation of these compounds from *Weigela subsessilis* L. H. Bailey. In addition, three compounds **7**, **9**, and **12** were found to display a strong inhibitory effect on the production of IL-8 in the HT29 cells stimulated by TNF-α.

Key words: *Weigela subsessilis*, Sterols, Triterpenoids, Iridoid glycoside, TNF-α-induced IL-8 production

INTRODUCTION

The genus *Weigela*, a member of the family Caprifoliaceae, is comprised of roughly twelve species (Chang, 1997). All of these plants are widespread and cultivated specifically in Korea, Japan, and Northern China. Among them, four species *W. hortensis*, *W. praecox*, *W. florida*, and *W. subsessilis* have been found in Korea (Chang, 1997).

W. subsessilis is a Korean endemic deciduous shrub, 2-4 m tall (Kim, 2004b). The plant has opposite leaves and yellowish green flowers that bloom on axils in spring, then change to red. *W. subsessilis* grows on sunny mountainous districts. Although this plant is widespread all over Korea, it has been rarely reported for use in folk medicine. Recent phytochemical studies of the leaves of this plant has resulted in the isolation of flavonoids and coumarins (Won *et al.*, 2004; Thuong *et al.*, 2005). The flavonoids from this plant were reported as kaempferol-*O*-3-α-L-(3-*O*-acetyl) rhamnopyranosyl-7-*O*-α-L-rhamnopyranoside sutchuenoside A, kaempferitrin, astragalin, kaempferol 7-*O*-rhamnoside, and kaempferol 3-*O*-α-L-rhamnosyl-7-*O*-β-D-glucoside. Four coumarins, scopoletin, cleomiscosin A, scopolin, and fraxin, were also isolated from these leaves. But to date, there has been no further work reported on other chemical compositions from this plant. In the present paper, the isolation, structural elucidation of twelve compounds from this plant and their inhibitory effect on IL-8 production in the HT29 cells induced by TNF-α are reported.

MATERIALS AND METHODS

Plant material

W. subsessilis (leaf and stem) was collected in April, 2000 at Gyeryong Mountain, Chungnam Province, Korea and identified by Prof. KiHwan Bae, one of the authors. A voucher specimen (CNU 2009) has been deposited in the herbarium at the College of Pharmacy, Chungnam National University, Daejeon, Korea.

General experimental procedures

Melting point (m.p.) determinations were performed using

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a Kofler micro-hotstage. IR spectra (KBr) were obtained on a Bruker spectrophotometer. FAB-MS and HR-FAB-MS were registered using a JEOL JMS-DX 300 spectrometer. NMR spectra including ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) were recorded on a Bruker DRX-300 NMR spectrometer. Analytical TLC were performed on pre-coated silica gel 60 F_{254} plates (Merck) or RP-18 F_{254}. For column chromatography, silica gel (Kieselgel 60, Merck), Sephadex LH-20 (Amersham Biosciences), and C-18 (Merck) were used.

Isolation of compounds

Dried and powdered plant material (6.7 kg) was extracted with methanol at room temperature (50 L × 3 times) for one month. The methanol extracts were combined and concentrated *in vacuo* to give a residue (684 g). The residue was suspended in water and partitioned successively with hexane, EtOAc, and BuOH and then exhaustively evaporated to yield a hexane fraction (90 g), an EtOAc fraction (163 g), a BuOH fraction (164 g) and a water fraction, respectively. The hexane fraction was chromatographed on a silica gel column using hexane-EtOAc (50:1 → 1:1) as the eluting solvent and separated into 6 subfractions (1-6). Subfraction 4 (8 g) was rechromatographed on a silica gel column eluted with hexane-EtOAc (10:1) to give compound **1** (34 mg) and **2** (117 mg). Subfraction 5 (12 g) was rechromatographed on a silica gel column eluted with hexane-EtOAc (1:5) to give 4 subfractions (5.1-5.4). Then, compound **3** was obtained after crystallizing out from subfraction 5.3 using the eluted solvent system. The EtOAc fraction (163 g) was subjected to column chromatography on a silica gel eluted with hexane-acetone (10:1 to 0:1) to give 8 fractions (fr. EA.1-8). Fraction EA.1-2 (49 g) was further chromatographed on a silica gel column eluted with hexane-EtOAc (1:4) to give compound **4** (323 mg). Fr. EA.3 (15 g) was rechromatographed on a Sephadex LH-20 eluted with MeOH-H_2O (20:1) to yield compound **4** (32 mg) and **5** (15 mg). The residue of fraction 3 was subjected to a C-18 column chromatography eluted with MeOH-H_2O (20:1) to yield compound **6** (181 mg) and **7** (234 mg). Compound **8** (6.7 mg) was obtained from fraction 4 (9 g) after C-18 column chromatography eluted with MeOH-H_2O (20:1). Fraction 6 (33 g) was rechromatographed on a C-18 column eluted with MeOH-H_2O (2:1) to yield compound **9** (7.1 mg) and **10** (21 mg). The fraction EA.8 (21 g) was separated by Sephadex LH-20 eluted with MeOH-H_2O (1:1), which gave compound **11** (23 mg). The BuOH fraction was chromatographed on a silica gel column using $CHCl_3$-MeOH-H_2O (80:20:1) as the eluting solvent and separated into 3 fractions (Fr. Bu.1-3). Finally, compound **12** (230 mg) was afforded from Fr. Bu.2 (41 g) after subjecting this fraction to a Sephadex LH-20 column chromatography eluted with MeOH-H_2O (1:2).

Squalene (1)
Colorless oil; FAB-MS *m/z* 433 [M+Na]$^+$; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 5.16 (6H, m, H-4, 9, 14), 2.10 (20H, m, CH_2), 1.70 (6H, s, H-2), 1.62 (18H, s, H-1, 8, 13); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 135.4 (C-12 and C-7), 131.6 (C-1), 124.8 (C-4), 124.7 (C-14 and C-9), 40.1 (C-6 and C-11), 28.6 (C-15), 27.1 (C-5 and C-10), 26.0 (C-1), 18.0 (C-3), 16.4 (C-8 and C-13).

β-Sitosterol acetate (2)
Colorless oil; IR ν_{max} ($CHCl_3$) cm^{-1}: 1720 (C=O), 1640 (C=C), 1060 (C-O); ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 5.38 (1H, t, *J* = 5.4 Hz, H-6), 4.62 (1H, m, H-3), 2.05 (3H, s, $OCOCH_3$), 1.04 (3H, s, H-19), 0.87 (3H, s, H-21), 0.84 (3H, t, *J* = 6.6 Hz, H- 29), 0.70 (3H, s, H-18); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 170.9 (O-C=O), 140.1 (C-5), 123.0 (C-6), 74.4 (C-3), 57.1 (C-14), 56.5 (C-17), 50.5 (C-9), 46.3 (C-24), 42.7 (C-13), 40.1 (C-12), 38.5 (C-4), 37.4 (C-1), 36.9 (C-10), 36.5 (C-20), 34.4 (C-22), 32.3 (C-7 and C-8), 29.6 (C-27), 28.6 (C-2), 28.2 (C-16), 26.6 (C-23), 24.7 (C-15), 23.5 (C-25), 21.8 (H_3C-COO), 21.4 (C-11), 20.2 (C-21), 19.7 (C-26), 19.4 (C-19), 19.1 (C-28), 12.4 (C-29), 12.2 (C-18).

β-Sitosterol (3)
Colorless needle; m.p. 140-141°C; IR ν_{max} (KBr) cm^{-1}: 3320 (OH), 1642 (C=C), 1050 (C-O); ^{1}H-NMR and ^{13}C-NMR were in accordance with authentic data (Chang *et al.*, 1981).

Ursolic acid (4)
Amorphous powder; m.p. 260-262°C; IR λ_{max} (KBr) cm^{-1} 3400 (OH), 2920, 1680 (C=O), 1450, 1380, 1185 (C-O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 5.11 (1H, s, H-12), 4.28 (1H, s, H-3), 2.98 (1H, s, H-18), 1.02, 0.88, 0.85, 0.73, 0.66 (each 3H, s, H-23, 27, 26, 24, 25), 0.89 (3H, d, *J* = 7.6 Hz, H-30), 0.80 (3H, d, *J* = 6.4 Hz, H-29); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 178.1 (C-28), 138.0 (C-13), 124.4 (C-12), 76.7 (C-3), 54.7 (C-5), 52.3 (C-18), 47.0 (C-17), 46.8 (C-9), 41.6 (C-14), 38.5 (C-19), 38.4 (C-8 and C-20), 38.3 (C-10), 38.2 (C-1), 36.5 (C-4), 36.3 (C-22), 32.7 (C-7), 30.2 (C-21), 28.3 (C-23), 27.5 (C-15), 27.0 (C-2), 23.8 (C-16), 23.3 (C-27), 22.9 (C-11), 21.1 (C-30), 18.0 (C-6), 17.0 (C-26), 16.9 (C-29), 16.1 (C-25), 15.3 (C-24).

Ilekudinol A (5)
Amorphous powder; m.p. 169-171°C; IR ν_{max} (KBr) cm^{-1}: 3430 (OH), 2959, 2929 and 2859, 1727 (C=O), 1275; FAB-MS *m/z* 477 [M+Na]$^+$ and 455 [M+H]$^+$; ^{1}H-NMR (300 MHz, CD_3OD) δ (ppm): 6.10 (1H, d, *J* = 10.8 Hz, H-11), 5.64 (1H, dd, *J* = 10.2, 3.0 Hz, H-12), 5.19 and 4.77 (each 1H, s, H-23), 3.75 (1H, d, *J* = 9.0 Hz, H-3), 3.50 (1H, m, H-2), 2.30 (1H, m H-15), 2.24 (1H, s, H-5), 2.20 (1H, dd, *J* = 12.6, 4.8 Hz, H-1b), 1.78 (1H, d, *J* = 10.8 Hz, H-18),

1.29 (3H, s, H-26), 1.09 (1H, d, J = 12.0 Hz, H-1a), 1.05 (3H, s, H-27), 1.04 (3H, d, J = 6.0 Hz, H-29), 0.97 (3H, d, J = 7.8 Hz, H-30), 0.76 (3H, s, H-25); ^{13}C-NMR (75 MHz, CD_3OD) δ (ppm): 181.5 (28), 150.1 (C-4), 133.6 (C-11), 129.0 (C-12), 104.4 (C-23), 90.8 (C-13), 78.5 (C-3), 73.0 (C-2), 60.8 (C-18), 50.8 (C-5), 49.8 (C-9), 46.5 (C-1), 45.6 (C-17), 42.3 (C-14), 41.9 (C-8), 40.5 (C-19), 38.3 (C-20), 37.8 (C-10), 31.5 (C-22), 30.7 (C-21), 29.8 (C-7), 25.6 (C-15), 22.8 (C-16), 20.6 (C-6), 18.4 (C-27), 18.2 (C-30), 17.2 (C-29), 15.5 (C-26), 15.4 (C-25).

Corosolic acid (6)

Amorphous powder; m.p. 274-276°C; IR ν_{max} (KBr) cm^{-1}: 3430 (OH), 2927, 1692 (C=O), 1565 and 1462(C=C), 1273 and 1049 (C-O); FAB-MS *m/z* 495 [M+Na]$^+$; ^{1}H-NMR (300 MHz, CD_3OD) δ (ppm): 5.24 (1H, t, J = 6.6 Hz, H-12), 3.63 (1H, m, H-2), 2.93 (1H, d, J = 9.6 Hz, H-3), 2.21 (1H, d, J = 11.4 Hz, H-18), 2.03 (1H, td, J = 13.2, 3.6 Hz, H-16a), 1.96 (1H, t, J = 3.6 Hz, H-1), 1.93 (1H, dd, J = 12.6, 4.8 Hz, H-15), 1.71 (1H, d, J = 3.6 Hz, H-22), 1.67 (1H, dd, J = 17.4, 3.6 Hz, H-22), 1.65 (1H, m, H-16b), 1.60 (1H, H-9), 1.58 (1H, m, H-7), 1.56 (1H, m, H-6), 1.51 (1H, m, H-21), 1.40 (1H, m, H-6), 1.35 (1H, m, H-21), 1.14 (3H, s, H-27), 1.08 (1H, d, J = 12.6 Hz, H-15), 1.01 (6H, s, H-23 and H-25), 0.96 (3H, d, J = 6.1 Hz, H-30), 0.89 (3H, d, J = 6.4 Hz, H-29), 0.85 (3H, s, H-26), 0.84 (1H, s, H-5), 0.81 (3H, s, H-24); ^{13}C-NMR (75 MHz, CD_3OD) δ (ppm): 181.1 (C-28), 139.9 (C-13), 124.5 (C-12), 83.1 (C-3), 68.0 (C-2), 55.7 (C-5), 53.6 (C-18), 48.0 (C- 9 and C-17), 47.8 (C-1), 42.6 (C-14), 39.9 (C-4), 39.8 (C-8), 39.5 (C-19), 39.4 (C-20), 38.5 (C-10), 37.6 (C-22), 33.7 (C-8), 31.5 (C-21), 29.7 (C-23), 28.6 (C-15), 25.1 (C-16), 24.1 (C-27), 23.8 (C-11), 22.2 (C-30), 18.9 (C-6 and C-26), 18.0 (C-29), 17.3 (C-24 and C-25).

Ilekudinol B (7)

Amorphous powder; m.p. 193-195°C; IR ν_{max} (KBr) cm^{-1}: 3431 (OH), 2926, 1693 (C=O); FAB-MS *m/z* 479 [M+Na]$^+$; ^{1}H-NMR (300 MHz, CD_3OD) δ (ppm): 5.17 (1H, br s, C-12), 5.10 and 4.59 (each 1H, s, H-23), 3.57 (1H, d, J = 8.7 Hz, H-3), 3.28 (1H, m, H-2), 2.18 (1H, d, J = 10.8 Hz, H-18), 1.08 (3H, s, H-27), 0.93 (3H, br s, H-30), 0.84 (3H, d, J = 6.3 Hz, H-29), 0.80 (3H, s, H-26), 0.69 (3H, s, H-25); ^{13}C-NMR (75 MHz, CD_3OD) δ (ppm): 179.1 (C-28), 152.0 (C-4), 139.4 (C-13), 125.5 (C-12), 104.9 (C-23), 78.6 (C-3), 72.8 (C-2), 53.4 (C-18), 50.3 (C-5), 48.1 (C-1), 47.7 (C-17), 45.2 (C-9), 42.8 (C-14), 39.4 (C-20), 39.3 (C-19), 38.7 (C-8 and C-10), 37.2 (C-22), 32.0 (C-7), 31.1 (C-21), 28.3 (C-15), 24.7 (C-11 and C-16), 21.5 (C-6), 24.1 (C-27), 21.9 (C-30), 17.9 (C-29), 17.8 (C-26), 15.6 (C-25).

Esculentic acid (8)

Amorphous powder; IR ν_{max} (KBr) cm^{-1}: 3433 (OH), 2937, 1692 (C=O), 1565 (C=C), 1049 (C-O); FAB-MS *m/z* 511 [M+Na]$^+$; ^{1}H-NMR (300 MHz, CD_3OD) δ (ppm): 5.16 (1H, t, J = 6.6 Hz, H-12), 4.32 (1H, d, J = 2.4 Hz, H-2), 3.73 (1H, d, J = 2.4 Hz, H-3), 3.36 and 3.22 (each 1H, d, J = 1.8 Hz, H-23), 2.12 (1H, d, J = 11.4 Hz, H-18), 1.35 (1H, m, H-5), 1.04 (3H, s, H-27), 0.94 (3H, s, H-25), 0.93 (3H, d, J = 6.6 Hz, H-30), 0.83 (3H, d, J = 6.6 Hz, H-29), 0.75 (3H, s, H-26), 0.73 (3H, s, H-24); ^{13}C-NMR (75 MHz, CD_3OD) δ (ppm): 179.1 (C-28), 139.1 (C-13), 125.4 (C-12), 76.8 (C-3), 70.0 (C-23), 65.6 (C-2), 53.3 (C-18), 47.8 (C- 9), 47.7 (C-17), 44.1 (C-5), 42.6 (C-14), 42.5 (C-1), 42.0 (C-4), 39.6 (C-8), 39.3 (C-19), 39.2 (C-20), 38.4 (C-10), 37.1 (C-22), 33.2 (C-7), 31.0 (C-21), 28.3 (C-15), 24.7 (C-16), 24.2 (C-27), 23.7 (C-11), 21.9 (C-30), 18.3 (C-6), 17.9 (C-29 and C-24), 17.8 (C-26), 17.5 (C-25).

Pomolic acid (9)

Amorphous powder; IR ν_{max} (KBr) cm^{-1}: 3430 (OH), 2930, 2876, 1691 (C=O), 1385, 1046; FAB-MS *m/z* 473 [M+H]$^+$; ^{1}H-NMR (300 MHz, CD_3OD) δ (ppm): 5.60 (1H, t, J = 4.5 Hz, H-12), 4.91 (1H, s, H-3), 1.72, 1.46, 1.22, 1.10, 1.01 and 0.91 (each 3H, s, H-23, 24, 25, 26, 27, 29), 1.10 (3H, d, J = 6.3 Hz, H-30); ^{13}C-NMR (75 MHz, CD_3OD) δ (ppm): 180.9 (C-28), 140.1 (C-13), 128.2 (C-12), 78.4 (C-3), 72.9 (C-19), 56.1 (C-5), 54.8 (C-18), 48.5 (C-17), 48.0 (C-9), 42.5 (C-20), 42.3 (C-14), 40.5 (C-8), 39.5 (C-4), 39.1 (C-1), 38.6 (C-22), 37.5 (C-10), 33.8 (C-7), 30.1 (C-15), 29.5 (C-2), 28.9 (C-23), 27.3 (C-29), 27.1 (C-21), 26.5 (C-16), 24.9 (C-27), 24.2 (C-11), 19.1 (C-6), 17.4 (C-26), 16.9 (C-30), 16.7 (C-25), 15.7 (C-24).

Asiatic acid (10)

Amorphous powder; m.p. 295-297°C; IR ν_{max} (KBr) cm^{-1}: 3410 (OH), 2920, 2875, 1690 (C=O), 1370 (C=C), 1050; ^{1}H-NMR (300 MHz, CD_3OD) δ (ppm): 5.45 (1H, t, J = 4.5 Hz, H-12), 4.18 (3H, m, H-23 and H-23), 3.72 (1H, d, J = 10.5 Hz, H-3), 2.59 (1H, d, J = 11.1 Hz, H-18), 1.12 (3H, s, H-27), 1.04 (6H, s, H-25, 26), 0.94 (3H, d, J = 6.6 Hz, H-30), 0.91 (3H, d, J = 6.0 Hz, H-29); ^{13}C-NMR (75 MHz, CD_3OD) δ (ppm): 180.1 (C-28), 139.5 (C-13), 125.7 (C-12), 78.4 (C-3), 69.0 (C-2), 66.7 (C-23), 53.7 (C-18), 48.2 (C-17), 48.2 (C-9), 48.1 (C-5), 43.8 (C-14), 42.7 (C-4), 40.2 (C-8), 39.6 (C-20 and C-19), 38.5 (C-10), 37.6 (C-22), 33.3 (C-7), 31.2 (C-15), 28.8 (C-16), 25.0 (C-21), 24.0 (C-27), 23.9 (C-11), 21.5 (C-26), 18.7 (C-6), 17.7 (C-30 and C-29), 17.6 (C-25), 14.5 (C-24).

Daucosterol (11)

Colorless crystal; m.p. 280-282°C; IR ν_{max} (KBr) cm^{-1}: 3391 (OH), 2361, 1635 (C=C), 1107 (C-O); ^{1}H-NMR and ^{13}C-NMR were in accordance with authentic data (Chang *et al.*, 1981).

Alboside I (12)

Amorphous powder; IR ν_{max} (KBr) cm^{-1}: 3419 (OH), 2935 (C-H), 1690 (C=O), 1631 (C=C), 1272, 1161 (C-O), 1073; FAB-MS *m/z* 553 $[M + H]^+$; ^{1}H-NMR (300 MHz, CD_3OD) δ (ppm): 7.57 (1H, d, *J* = 15.9 Hz, H-7), 7.38 (1H, d, *J* = 1.2 Hz, H-3), 7.33 (1H, d, *J* = 1.8 Hz, H-2), 7.12 (1H, dd, *J* = 1.8, 8.4 Hz, H-6), 6.80 (1H, d, *J* = 8.4 Hz, H-5), 6.48 (1H, d, *J* = 15.9 Hz, H-8), 5.18 (1H, d, *J* = 5.4 Hz, H-1), 5.17 (1H, m, H-2), 4.53 (1H, d, *J* = 7.8 Hz, H-1), 3.83 (3H, s, OCH_3), 3.70-2.90 (6H, m, H-glucose), 2.22 (1H, m, H-6a), 2.10 (1H, m, H-8), 1.98 (1H, m, H-9), 1.74 (1H, m, H-6b), 1.02 (3H, d, *J* = 6.6 Hz, H-10); ^{13}C-NMR (75 MHz, CD_3OD) δ (ppm): 168.8 (C-11), 167.2 (C-9), 151.5 (C-3), 150.2 (C-3), 148.8 (C-4), 145.9 (C-8), 126.5 (C-1), 124.1 (C-5), 116.3 (C-2), 115.5 (C-7), 112.5 (C-4), 112.0 (C-6), 99.6 (C-1), 96.7 (C-1), 78.1 (C-5), 77.6 (C-7), 77.1 (C-3), 74.1 (C-2), 71.0 (C-4), 62.1 (C-6), 56.6 (OCH_3), 46.1 (C-9), 40.6 (C-8), 40.3 (C-6), 32.2 (C-5), 14.4 (C-10).

Inhibitory effect of IL-8 secretion assay

Cell line, cell culture and enzyme-linked immunosorbent assay (ELISA) for IL-8 production from HT29 cells were performed accordingly to the reported method (Kim *et al.*, 2004a).

RESULTS AND DISCUSSION

The present phytochemical study of MeOH extract resulted in the isolation of twelve compounds, including three from the hexane fraction **1**, **2**, and **3**, eight from the EtOAc fraction **4**, **5**, **6**, **7**, **8**, **9**, **10**, and **11**, and one from the BuOH fraction **12**.

Compound **1** was obtained as colorless oil. The molecular formula was determined as $C_{30}H_{50}$ by the peak $[M+H]^+$ at *m/z* 411 in FAB-MS. ^{13}C-NMR and DEPT showed signals due to three methyls at δ 16.4, 18.0, and 26.0; three methylenes at δ 27.1, 28.6, and 40.1, two methines at δ 124.7, 124.8 and two quaternary carbons at δ 135.2 and 135.5. In addition, the ^{1}H-NMR spectrum demonstrated that **1** possessed vinylic protons at δ 5.16 (6H, m), and allylic protons appeared at brood multiple signals at δ 2.10 (20 H) and two singlet signals at δ 1.70 and 1.62. All of the above evidences suggest that **1** had eight methyl, ten methylene, and six methine groups along with six quaternary carbons. A detailed comparison of ^{1}H-NMR and ^{13}C-NMR spectral data with those reported in literature concluded that **1** was squalene (Burger *et al.*, 1978).

Two compounds, **3** and **11**, were easily deduced to be β-sitosterol and β-sitosterol glucoside (daucosterol) by direct comparison of spectral data with those reported in literature (Chang *et al.*, 1981). Compound **2** was isolated as colorless oil. The IR, ^{1}H-NMR, and ^{13}C-NMR spectra were closely similar to those of **3.** The ^{1}H-NMR spectrum displayed a singlet peak at 2.05 (3H, s) and ^{13}C-NMR showed peaks at δ 21.8 and 170.9, which all certified an acetate group (CH_3COO). Thus, **2** was identified as β-sitosterol acetate.

Seven compounds **4**, **5**, **6**, **7**, **8**, **9**, and **10** were all obtained as amorphous powder and showed positive reactions with triterpenoids detectors, Carr-Price and Liebermann-Burchard reagents. In addition, the IR spectra of these compounds all revealed the presence of both hydroxyl (OH) and carbonyl (C=O) groups in the molecule, suggesting that they were triterpenoids. Additional detailed comparisons of spectroscopic data with literature values will further support this suggestion.

The m.p. 260-262°C of compound **4** resembled that of ursolic acid, which has been previously reported from *W. coraeensis* (Iwagawa and Hase, 1988). The comparison of ^{1}H-NMR and ^{13}C-NMR with those in literature (Na *et al.*, 2002) supported this observation. By means of similar method, compound **6** was identified as corosolic acid (Taniguchi *et al.*, 2002).

Compound **5** had an m.p. of 169-171°C. The ^{1}H-NMR spectrum of **5** showed two double peaks at δ 6.10 and 5.64 and two singlet peaks at δ 5.19 and 4.77, all of which came from olefinic protons. In addition, the ^{13}C-NMR spectrum displayed four olefinic carbons at δ 150.1, 133.6, 129.0, and 104.4, suggesting the existence of two double bonds in the molecule. The most downfield peak at δ 181.5 revealed the presence of a carbonyl group. There were two oxygenated methine carbon peaks at δ 78.5 and 73.0, demonstrating the appearance of two hydroxyl groups. The coupling constant (*J* = 9.0 Hz) of two protons at δ 3.75 (1H, *J* = 9.0 Hz) and 3.50 (1H, m) indicated the *trans*-form. Thus, of the two protons at C-2 and C-3, one was the a-form (H-3) and the other was the β-form (H-2). Five methyl resonances were shown in both ^{1}H-NMR and ^{13}C-NMR. Among them, two revealed a coupling proton at 1.04 (3H, d, *J* = 6.0 Hz) and 0.97 (3H, d, *J* = 7.8 Hz). Therefore, compound **5** was deduced to be an ursane-triterpenoid. Additionally, the molecular weight of **5** was 454, given by the peak $[M+H]^+$ at *m/z* 455 in FAB-MS. All of these evidences led to conclusion that **5** was ilekudinol A, which was originally reported from *Ilex kudingcha* (Nishimura *et al.*, 1999).

The structure of compound **7** was similar to that of **5**, as evidenced by the patterns of ^{1}H-NMR and ^{13}C-NMR spectra. However, there were three olefinic protons that showed at δ 5.17 (1H, br s), 5.01 and 4.59 (each 1H, s). One olefinic methylene carbon appeared at δ 104.9, which possessed two singlet protons at δ 5.01 and 4.59. There were two hydroxyl groups in the molecule that were revealed by the appearance of two oxygenated methine

carbons at δ 78.6 and 72.8. The FAB-MS spectrum showed the peak $[M+Na]^+$ at *m/z* 479, giving the molecular weight 456. All of these data resembled those of ilekudinol B, which has been reported to be present in *Ilex kudingcha* (Nishimura *et al.*, 1999). Additional comparisons of the spectral data with those published in literature elucidated that **7** was ilekudinol B.

Compound **8** showed the molecular peak $[M+H]^+$ at *m/z* 489 in FAB-MS, suggesting the molecular formula $C_{30}H_{48}O_5$. The ^{1}H-NMR and ^{13}C-NMR spectra, which showed triterpenoid patterns, indicated one double bond at δ 5.16 of the proton at δ 139.1 and 125.4 of the carbon. The peak at δ 179.1 revealed a carboxyl group in the molecule. Among the six methyl groups that appeared in the spectrum, two showed coupling with another proton, thus establishing the ursane-triterpenoids skeleton. The methylene peak at δ 70.0, along with two other oxygenated methine carbons at δ 76.8 and 65.6 suggested that **8** possessed three hydroxy groups. Two coupling protons at δ 4.32 (1H, d, J = 2.4 Hz) and 3.73 (1H, d, J = 2.4 Hz) indicated the *cis*-form. Oxymethylene signals were also observed at δ 3.36 and 3.22 which showed the coupling constant J = 1.8 Hz. Taken together, one hydroxy group was deduced to be at C-23 and the two others were a-form at C-2 and C-3. Therefore, **8** was identified as esculentic acid (Ahmad *et al.*, 1986).

Compound **9** had the molecular weight of 472, given by the peak $[M+H]^+$ at *m/z* 473 in FAB-MS. The ^{1}H-NMR and ^{13}C-NMR spectra demonstrated that **9** was possibly another ursane-triterpenoid with the molecular formula $C_{30}H_{48}O_4$. But only one of seven methyl groups experienced coupling with another proton. However, the quaternary carbon at δ 72.9 revealed that it contact to hydroxyl group, indicating that **9** was probably pomolic acid. Further support was obtained by comparison with published data (Cheng and Cao, 1992). Therefore, **9** was assigned as pomolic acid.

The ^{1}H-NMR and ^{13}C-NMR spectra of compound **10** also indicated that it was most likely an ursane-triterpenoid. From the ^{1}H-NMR spectrum, two doublet signals appeared at δ 0.94 (3H, d, J = 6.6 Hz) and 0.91 (3H, d, J = 6.0 Hz) due to methyl groups at C-19 and C-20. The proton at C-18 appeared at δ 2.59 (1H, d, J = 11.1 Hz). The peak at 5.45 (1H, t, J = 4.5 Hz) was probably due to an olefinic proton at C-12 (d 125.7). The methylene signal peak at δ 66.7 and two methines at δ 78.4 and 69.0 revealed three hydroxyl groups in the molecule. In addition, two protons at 4.18 (1H, m) and 3.72 (1H, d, J = 10.5 Hz) indicated the *trans*-form. Thus, two hydroxy groups were probably at C-2 (α-form) and C-3 (β-form), and the other contact with C-23. After additional comparison of ^{1}H-NMR and ^{13}C-NMR spectral data with those in literature (Furuya *et al.*, 1987),

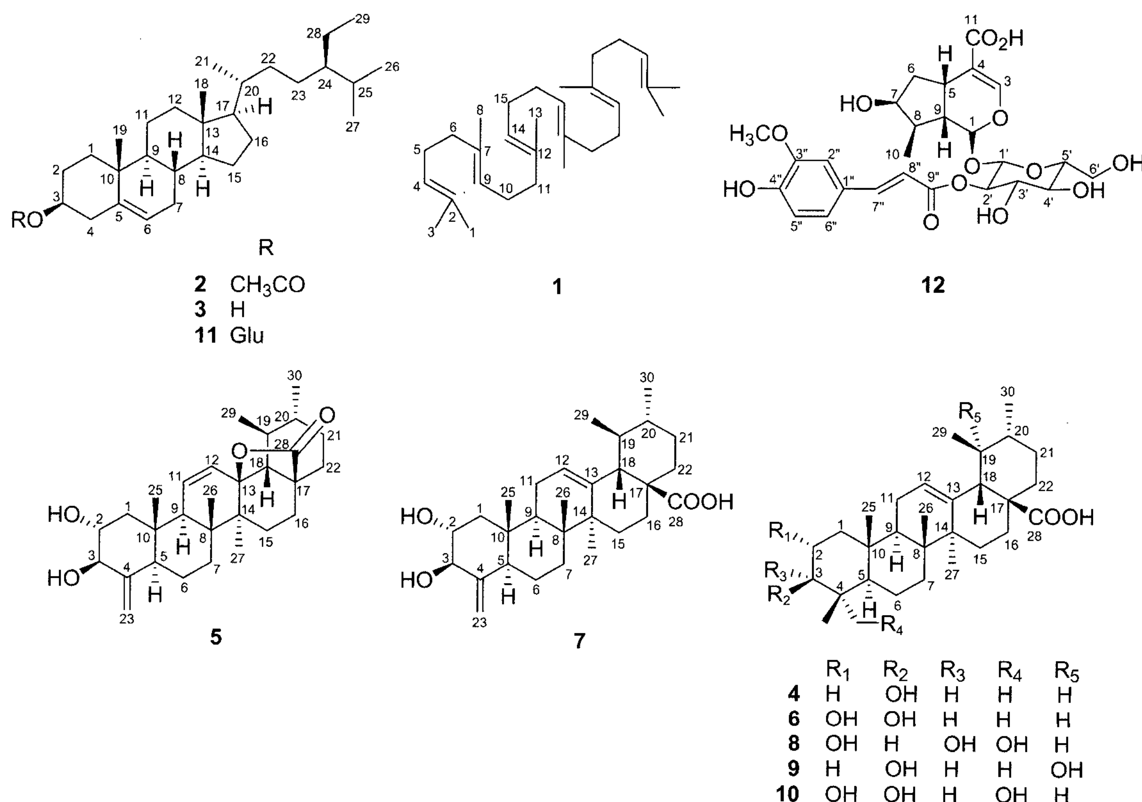

Fig. 1. Chemical structures of compounds (**1-12**) isolated from *W. subsessilis*

Table I. Inhibitory effect of active compounds on TNF-α-stimulated IL-8 secretion from HT29 cell line

Compounds	Inhibition[a] (%)		
	10 (μg/mL)	1 (μg/mL)	0.1 (μg/mL)
4	35 ± 2.3	17 ± 1.4	0
5	30 ± 2.1	26 ± 2.6	11 ± 1.0
6	23 ± 1.4	21 ± 1.2	7 ± 0.2
7	52 ± 3.0*	25 ± 1.4	11 ± 0.9
8	23 ± 1.2	22 ± 1.2	21 ± 1.3
9	48 ± 2.5*	39 ± 2.1*	27 ± 1.5
10	31 ± 2.0	12 ± 1.3	0
12	41 ± 2.1*	24 ± 1.2	10 ± 1.4
Sulfasalazine[a]		72 ± 4.2*	27 ± 1.8

[a]The results were calculated from four separated experiments (*$p < 0.05$).
[b]Sulfasalazine was used as a positive control.

10 was identified as asiatic acid.

Compound **12**, which was obtained as amorphous powder, had a molecular mass 552 and the molecular formula $C_{26}H_{32}O_{13}$, given by the peak $[M+H]^+$ at *m/z* 553 in FAB-MS. The IR spectrum showed some considerable peaks at 3419 (OH), 1690 (C=O), 1631 (C=C), and 1161 (C-O). ^{1}H-NMR displayed two doublets at δ 7.57 and 6.48 with the coupling constant of 15.9 Hz, attributed to a *trans*-α, β-unsaturated carbonyl function. The appearance of two doublets at δ 7.33 (1H, d, J = 1.8 Hz) and 6.80 (1H, d, J = 8.4 Hz), and the double doublets at δ 7.12 (1H, dd, J = 1.8, 8.4 Hz) revealed the aromatic ring that was 1, 3, 4-trisubstituted. The peak at δ 3.83 (3H, s) was resulted from a methoxy group (OCH_3), which was also shown in ^{13}C-NMR at δ 56.6. Therefore, feruloyl moiety was a part of the molecule. Moreover, both ^{1}H-NMR and ^{13}C-NMR showed the presence of glucose with the appearance of anomeric protons and carbons. Futhermore, both spectra also demonstrated the existence of loganin moiety by proton peaks at δ 7.38 (1H, d, J = 1.2 Hz), 2.22 (1H, m), 2.10 (1H, m), and 1.98 (1H, m); the carbon signal at δ 168.8 might possibly result from the carbon of a carboxyl group, and the peak at δ 14.4 from a methyl group (CH_3). All of these evidences led to the conclusion that **12** was alboside I, which was first reported from *Chioccoca alba* (Carbonezi *et al.*, 1999).

Among the isolates, seven ursane-type triterpenoids **4**, **5**, **6**, **7**, **8**, **9**, and **10** and an iridoid glycoside **12** showed positive inhibitory effect on IL-8 secretion from HT29 cell line induced by TNF-α (Table I). Of which, three compounds **7**, **9**, and **12** exhibited a strong activity. As shown in Table I, at concentration of 10 μg/mL, compound **7** disclosed the strongest inhibitory effect (52%) compare to those of **9** (48%) and **12** (41%). However, at the concentration of 1 and 0.1 μg/mL, **9** showed the most inhibition (39 and 27%, respectively). It is noteworthy that **9** displayed an equivalent activity to sulfasalazine, the compounds was used as a positive control, at the concentration of 0.1 μg/mL. This result shows that **7**, **9**, and **12** suppress TNF-α-induced IL-8 production and could be beneficial to inflammatory disorders.

The current paper reports the first isolation of twelve compounds from *W. subsessilis*. It is interesting to note that this is also the first report on the presence of nine compounds from *Weigela* species, including **1**, **2**, **5**, **6**, **7**, **8**, **9**, **10**, and **12**. The results, together with previous documents (Chang, 1997; Won *et al.*, 2004), demonstrate that ursane-triterpenoids and flavonoids are the major constituents of the title plant. Since triterpenoids have been documented to exhibit cytotoxicity (Chaturvedula *et al.*, 2004; Taniguchi *et al.*, 2002), antitumor activity (Taniguchi *et al.*, 2002), antidiabetes (Judy *et al.*, 2003; Miura *et al.*, 2004), hepatoprotectivity (Adnyana *et al.*, 2000; Liu *et al.*, 1994), anticomplementary (Oh *et al.*, 2000), antiinflammatory (Rajic *et al.*, 2001), and anti-AIDS activity (Kashiwada *et al.*, 1998), it is expected that *W. subsessilis* and its triterpenoids may possess numerous considerable bioactivities.

ACKNOWLEDGEMENT

This work was supported by a grant from BioGreen 21 Program (2005), Rural Development Administration, Republic of Korea.

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