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Pytochemical Constituents of the Aerial Parts from *Solidago virga-aurea* var. *gigantea*

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The chromatographic separation of the MeOH extract of the aerial parts of *Solidago virga-aurea* var. *gigantea* M_{IQ}. (Compositae) led to the isolation of six terpenoids and four phenolic compounds, *trans*-phytol (**1**), *ent*-germacra-4(15),5,10(14)-trien-1α-ol (**2**), β-amyrin acetate (**3**), *ent*-germacra-4(15),5,10(14)-trien-1β-ol (**4**), β-dictyopterol (**5**), oleanolic acid (**6**), kaempferol (**7**), kaempferol-3-*O*-rutinoside (**8**), methyl 3,5-di-*O*-caffeoyl quinate (**9**), and 3,5-di-*O*-caffeoyl quinic acid (**10**). Their structures were established by chemical and spectroscopic methods. Compounds **4**, **5**, and **10** showed moderate cytotoxicity against five cultured human tumor cell lines *in vitro* with its ED_{50} values ranging from 1.52~18.57 μg/mL.

Key words: *Solidago virga-aurea* var. *gigantea* M_{IQ}, Compositae, Terpenoid, Flavonoid, Quinic acid, Cytotoxicity

INTRODUCTION

Solidago virga-aurea var. *gigantea* M_{IQ}. (Compositae), a perennial herb, is mainly distributed in the southern island of South Korea and especially cultivated as culinary vegetable in the Ullung island. This plant has been used as stomachic and diuretic in Korean folk medicine (Lee, 1979). Erythrodiol-3-acetate, α-tocopherol-quinone, *trans*-phytol and 2-methoxybenzyl-2,6-dimethoxybenzoate were reported from hexane-soluble fraction of this plant (Sung *et al.*, 1999). As part of our systematic study on the genus *Solidago* of Korean Compositae plants, we have investigated the constituents of *Solidago virga-aurea* var. *gigantea* M_{IQ}. As a result, six terpenoids and four phenolics, *trans*-phytol (**1**), *ent*-germacra-4(15),5,10(14)-trien-1α-ol (**2**), β-amyrin acetate (**3**), *ent*-germacra-4(15),5,10(14)-trien-1β-ol (**4**), β-dictyopterol (**5**), oleanolic acid (**6**), kaempferol (**7**), kaempferol-3-*O*-rutinoside (**8**), methyl 3,5-di-*O*-caffeoylquinate (**9**), and 3,5-di-*O*-caffeoylquinic acid (**10**) were isolated from the hexane-soluble fraction of the total extract. This paper describes the isolation, structural characterization and cytotoxic activities of the compounds.

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MATERIALS AND METHODS

General experimental procedure

Mps: uncorr. Optical rotations: Jasco P-1020 Polarimeter. NMR: Bruker AMX 500 and Varian UNITY INOVA 500. IR: in CCl_4, Nicolet model 205 FT-IR spectrophotometer. MS: VG70-VSEQ mass spectrometer. Column chromatography : Silica gel 60 (Merck, 70~230 mesh and 230~400 mesh), Licroprep. 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 (240×10 mm).

Plant materials

The aerial parts of *Solidago virga-aurea* var. *gigantea* (Compositae) were collected at Ullung island, Korea in August, 2001. A voucher specimen (SKK-01-014) was deposited at the College of Pharmacy in SungKyunKwan University.

Cytotoxicity testing

Sulforhodamin B Bioassay (SRB) was used for cytotoxicity evaluation. The activity of a compound was tested at several concentration levels against five cultured human tumor cells *in vitro,* A549 (non small cell lung adenocar-

cinoma), 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 aerial parts of *Solidago virga-aurea* var. *gigantea* (2 kg) were extracted with MeOH (10 L) at room temperature. The resultant MeOH extract (300 g) was subjected to successive solvent partitioning to give *n*-hexane (60 g), CH_2Cl_2 (2.8 g), EtOAc (9 g), and *n*-BuOH (26 g) soluble fractions. The *n*-hexane extract (60 g) was chromatographed on a silica gel column using a gradient solvent system of *n*-hexane:EtOAc (10:0~0:1) to give five fractions (SH1~SH5). The fraction SH2 (3.5 g) was chromatographed on a silica gel column eluted with *n*-hexane:EtOAc (6:1) to give four subfractions (SH21~SH24). The subfraction SH21 (300 mg) was purified with Sephadex LH-20 (CH_2Cl_2:MeOH=1:1) and Lobar®-A column (*n*-hexane:EtOAc=7:1) to yield **1** (20 mg). The subfraction SH22 (600 mg) was purified with Sephadex LH-20 (CH_2Cl_2:MeOH=1:1) to give four subfractions (SH221~SH222). The second subfraction SH222 (250 mg) was purified with RP Lobar®-A column (80% MeCN) to yield **2** (12 mg). The subfraction SH24 (800 mg) was chromatographed on a silica gel column eluted with *n*-hexane:EtOAc (7:1), and then purified with Sephadex LH-20 (CH_2Cl_2:MeOH =1:1) and RP Lobar®-A column (80% MeCN) to yield **3** (20 mg). The fraction SH3 (7.5 g) was chromatographed on a silica gel column eluted with *n*-hexane:EtOAc (3:1) to give five subfractions (SH31~SH35). The subfraction SH33 (1.4 g) was chromatographed on Sephadex LH-20 (CH_2Cl_2:MeOH =1:1) to give five subfractions (SH331~SH335). The second subfraction SH332 (230 mg) was purified with RP Lobar®-A column (85% MeCN) to yield **4** (10 mg) and the fourth subfraction SH334 (150 mg) was purified with Lobar®-A column (*n*-hexane:EtOAc=3:1) to yield **5** (30 mg). The subfraction SH34 (2 g) was chromatographed on a silica gel column eluted with *n*-hexane:EtOAc (3:1), and then purified with Sephadex LH-20 (CH_2Cl_2:MeOH =1:1) to yield **6** (30 mg). The EtOAc extract (9 g) was chromatographed on a silica gel column using EtOAc:MeOH:H_2O (9:2:0.3) to give three fractions (SE1~SE3). The fraction SE1 (6 g) was chromatographed on silica gel column eluted with EtOAc: MeOH:H_2O (9:2:0.2) to give four subfractions (SE11~SE14).

The subfraction SE11 (1.2 g) was purified with Sephadex LH-20 (MeOH) and RP Lobar®-A column (50% MeOH) to yield **7** (10 mg). The subfraction SE12 (1.2 g) was chromatographed on a silica gel column eluted with EtOAc: MeOH:H_2O (9:2:0.5) and purified with Sephadex LH-20 (MeOH) and RP Lobar®-A column (50% MeOH) to yield **8** (15 mg) and **9** (15 mg). The subfraction SE13 (1.2 g) was purified with Sephadex LH-20 (MeOH) and RP Lobar®-A column (45% MeOH) to yield **10** (12 mg).

trans-Phytol (1)

Colorless oil, $[\alpha]_D$ +0.89° (c 0.30, $CHCl_3$); IR ($CHCl_3$) ν_{max}^{neat} cm^{-1} ν = 3443 (OH), 1667 (C=C); EI-MS *m/z* : 296 (M^+); ^{1}H-NMR (500 MHz, $CDCl_3$) : δ 5.42 (1H, tq like, *J* = 6.8, 1.2 Hz, H-2), 4.16 (2H, d, *J* = 6.8 Hz, H-1), 2.00 (2H, m), 1.68 (3H, s, H-3a), 1.64~1.02 (CH_2, CH), 0.88~0.85 (12H, m, H-7a, 11a, 15a, 16); ^{13}C-NMR (125 MHz, $CDCl_3$) : δ 140.6 (C-3), 123.3 (C-2), 59.7 (C-1), 40.1, 39.7, 37.7, 37.6, 37.5, 36.9, 33.1, 32.9, 28.2, 25.4, 25.1, 24.7, 23.0, 22.9, 20.01, 20.00, 16.4.

ent-Germacra-4(15),5,10(14)-trien-1α-ol (2)

Colorless oil, $[\alpha]_D$ +70.51° (c 0.20, $CHCl_3$); IR ($CHCl_3$) ν_{max}^{neat} cm^{-1} ν = 3415 (OH), 1680; EI-MS *m/z* : 220 (M^+); ^{1}H-NMR (500 MHz, $CDCl_3$) : δ 0.81 (3H, d, *J* = 7.0 Hz, H-13), 0.89 (3H, d, *J* = 7.0 Hz, H-12), 1.36 (1H, m, H-11), 1.45~2.03 (each 8H, m, H-8, 9, 7, 2 and 3), 2.40 (1H, br.dd, *J* = 4.5, 13.0 Hz, H-9), 3.78 (1H, m, H-1), 4.84 (1H, s, H-15), 4.95 (1H, s, H-15), 5.01 (1H, s, H-14), 5.27 (1H, s, H-14), 5.40 (1H, dd, *J* = 16.0, 10.5 Hz, H-6), 5.97 (1H, d, *J* = 16.0 Hz, H-5); ^{13}C-NMR ($CDCl_3$, 125 MHz) : δ 76.7 (C-1), 36.9 (C-2), 30.5 (C-3), 147.4 (C-4), 130.3 (C-5), 138.6 (C-6), 53.2 (C-7), 36.8 (C-8), 35.2 (C-9), 154.1 (C-10), 32.5 (C-11), 21.2 (C-12), 21.4 (C-13), 111.2 (C-14), 113.6 (C-15).

β-Amyrin acetate (3)

White powder, mp 230°C; EI-MS *m/z* : 468 (M^+); ^{1}H-NMR (500 MHz, $CDCl_3$) : δ 0.85 (3H, s, H-28), 0.88 (3H, s, H-23), 0.88~0.89 (9H, s, H-24, H-29 and H-30), 0.98 (3H, s, H-25), 0.99 (3H, s, H-26), 1.15 (3H, s, H-27), 2.06 (3H, s, $COCH_3$), 4.52 (1H, t like, *J* = 8.0 Hz, H-3a), 5.20 (1H, t, *J* = 3.5 Hz, H-12); ^{13}C-NMR (125 MHz, $CDCl_3$) : δ 15.6 (C-25), 16.7 (C-24), 16.8 (C-26), 18.3 (C-6), 21.3 ($COCH_3$), 23.5 (C-11), 23.7 (C-30), 23.7 (C-2), 25.9 (C-27), 26.2 (C-16), 26.9 (C-28), 28.0 (C-23), 28.3 (C-15), 31.2 (C-20), 32.5 (C-17), 32.6 (C-7), 33.3 (C-29), 34.7 (C-21), 36.9 (C-10), 37.1 (C-22), 37.8 (C-4), 38.2 (C-1), 39.8 (C-8), 41.7 (C-14), 46.8 (C-19), 47.3 (C-18), 47.7 (C-9), 55.2 (C-5), 80.9 (C-3), 121.7 (C-12), 145.2 (C-13), 171.0 (CO).

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

Colorless oil, $[\alpha]_D$ -40.21° (c 0.20, $CHCl_3$); IR ($CHCl_3$) ν_{max}^{neat} cm^{-1} ν = 3400 (OH), 1655; EI-MS *m/z* : 220 (M^+); ^{1}H-NMR (500 MHz, $CDCl_3$) : δ 0.83 (3H, d, *J* = 6.5Hz, H-13), 0.94 (3H, d, *J* = 6.5 Hz, H-12), 1.52~1.83 and 2.63 (6H, m, H-7, 8, 9 and 11), 2.08 (2H, m, H-2), 2.21 (1H, ddd, *J* = 13.0, 5.5, 3.0 Hz, H-3a), 2.48 (1H, m, H-3b), 3.80 (1H, dd, *J* = 12.0, 4.0 Hz, H-1), 4.86 (1H, br.s, H-15a), 4.95 (1H, br.s, H-15b), 5.01 (1H, br.s, H-14a), 5.30 (1H, br.s, H-14b), 5.44 (1H, dd, *J* = 16.0, 10.5 Hz, H-6), 6.04 (1H, d, *J* = 16.0 Hz, H-5); ^{13}C-NMR (125 MHz, $CDCl_3$) : δ 76.4 (C-1), 36.2 (C-2), 29.9 (C-3), 146.8 (C-4), 129.7 (C-5), 137.9 (C-6), 52.5 (C-7), 36.2 (C-8), 34.5 (C-9), 153.6 (C-10), 31.8 (C-

11), 20.7 (C-12), 20.5 (C-13), 112.9 (C-14), 110.5 (C-15).

β-Dictyopterol (5)
Colorless oil, $[\alpha]_D$ +20.68° (c 0.20, $CHCl_3$); IR ($CHCl_3$) ν_{max}^{neat} cm^{-1} ν = 3500 (OH), 2850, 1705, 1660; EI-MS *m/z* : 220 (M^+); ^{1}H-NMR (500 MHz, $CDCl_3$) : δ 0.70 (3H, s, H-14), 0.97 (1H, m, H-9), 1.31 (2H, m, H-6, 8), 1.50 (1H, m, H-2), 1.57 (2H, m, H-5, 6), 1.60 (2H, m, H-2, 8), 1.69 (3H, s, H-13), 1.82 (1H, tt, *J* = 11.0, 4.0 Hz, H-7), 1.90 (2H, m, H-3, 9), 2.18 (1H, m, H-3), 3.11 (1H, dd, *J* = 11.5, 4.5 Hz, H-1), 4.54 (1H, s, H-15), 4.75 (1H, br.s, H-15), 4.84 (1H, s, H-12), 4.86 (1H, s, H-12); ^{13}C-NMR (125 MHz, $CDCl_3$) : δ 79.0 (C-1), 31.8 (C-2), 34.5 (C-3), 148.5 (C-4), 47.8 (C-5), 26.7 (C-6), 45.1 (C-7), 28.9 (C-8), 37.0 (C-9), 40.5 (C-10), 150.3 (C-11), 108.1 (C-12), 20.9 (C-13), 10.5 (C-14), 106.8 (C-15).

Oleanolic acid (6)
White powder, mp 197°C; EI-MS *m/z* : 456 (M^+); ^{1}H-NMR (500 MHz, $CDCl_3$) : δ 0.74, 0.79, 0.89, 0.91, 0.92, 0.98 and 1.12 (each 3H, s, CH_3), 2.83 (1H, br. dd, *J* = 4.0, 14.0 Hz, H-18), 3.22 (1H, br. dd, *J* = 4.0, 9.5 Hz, H-3) and 5.28 (each 1H, m, H-12).

Kaempferol (7)
Yellow powder, mp 270°C; ^{1}H-NMR (500 MHz, CD_3OD) : δ 6.17 (1H, d, *J* = 2.0 Hz, H-6), 6.38 (1H, d, *J* = 2.0 Hz, H-8), 6.99 (2H, dd, *J* = 2.0, 8.5 Hz, H-3', 5'), 8.07 (2H, dd, *J* = 2.0, 8.5 Hz, H-2', 6').

Kaempferol-3-*O*-rutinoside (8)
Yellow powder, mp 180°C; ^{1}H-NMR (500 MHz, CD_3OD) : δ 1.11 (3H, d, *J* = 6.5 Hz, rha-CH_3), 4.51 (1H, br.s, rha-1), 5.12 (1H, d, *J* = 7.5 Hz, glc-1), 6.21 (1H, d, *J* = 2.0 Hz, H-6), 6.40 (1H, d, *J* = 2.0 Hz, H-8), 6.88 (2H, dd, *J* = 2.0, 7.0 Hz, H-3', 5'), 8.05 (2H, dd, *J* = 2.0, 7.0 Hz, H-2', 6').

Methyl 3,5-di-*O*-caffeoyl quinate (9)
Yellow gum, $[\alpha]_D$ -194.31° (c 0.20, MeOH); FAB MS *m/z*: 531 $[M+H]^+$; ^{1}H-NMR (500 MHz, CD_3OD) : δ 2.21 (brd, *J* = 12.5 Hz, H-2a), 1.99 (brd, *J* = 12.5 Hz, H-2b), 5.38 (br.dd, *J* = 9.5, 4.5 Hz, H-5), 3.98 (dd, *J* = 9.5, 3.0 Hz, H-4), 5.29 (dt, *J* = 7.0, 3.5 Hz, H-3), 1.98 (brd, *J* = 12.5 Hz, H-6), 6.23/6.14 (d, *J* = 16.0 Hz, H-2'), 7.48/7.42 (d, *J* = 16.0 Hz, H-3'), 7.04/7.04 (d, *J* = 2.5 Hz, H-5'), 6.77/6.77 (d, *J* = 8.0 Hz, H-8'), 6.99/6.99 (d, *J* = 8.0 Hz, H-9'), 3.59 (s, OCH_3); ^{13}C-NMR (125 MHz, CD_3OD) : δ 73.8 (C-1), 35.6 (C-2), 72.1 (C-3), 67.9 (C-4), 71.1 (C-5), 35.9 (C-6), 167.3 (C-1'), 166.8 (C-1"), 115.9 (C-2'), 115.8 (C-2"), 146.7 (C-3'), 146.6 (C-3"), 126.7 (C-4'), 126.4 (C-4"), 115.8 (C-5'), 114.8 (C-5"), 146.3 (C-6'), 146.1 (C-6"), 149.8 (C-7'), 149.5 (C-7"), 117.0 (C-8'), 116.9 (C-8"), 122.4 (C-9'), 122.3 (C-9"), 53.4 (OCH_3), 174.7 (CO).

3,5-Di-*O*-caffeoylquinic acid (10)
Yellow gum, $[\alpha]_D$ -230.14° (c 0.20, MeOH); FAB MS *m/z* : 517 $[M+H]^+$; ^{1}H-NMR (500 MHz, MeOD) : δ 2.11 (br.d, *J* = 12.5 Hz, H-2a), 1.87 (m, H-2b), 5.49 (dd. *J* = 9.9, 4.5 Hz, H-5), 3.41 (dd, *J* = 9.9, 2.8 Hz, H-4), 5.39 (br.dd, *J* = 6.5, 3.5 Hz, H-3), 1.90 (m, H-6), 6.24/6.22 (d, *J* = 15.5 Hz, H-2'), 7.47/7.46 (d, *J* = 15.5 Hz, H-3'), 7.06/7.05 (s, H-5'), 6.76/6.76 (d, *J* = 8.0 Hz, H-8'), 6.96/6.96 (d, *J* = 8.0 Hz, H-9'); ^{13}C-NMR (125 MHz, MeOD) : δ 75.6 (C-1), 37.0 (C-2), 73.7 (C-3), 71.8 (C-4), 72.2 (C-5), 40.3 (C-6), 167.3 (C-1"), 167.1 (C-1"), 117.2 (C-2'), 116.9 (C-2"), 145.7 (C-3'), 145.5 (C-3"), 126.7 (C-4'), 126.6 (C-4"), 115.9 (C-5') 115.6 (C-5"), 146.9 (C-6'), 146.8 (C-6"), 149.6 (C-7'), 149.4 (C-7"), 116.4 (C-8'), 116.2 (C-8"), 122.5 (C-9'), 122.1 (C-9"), 178.8 (COOH)

RESULTS AND DISCUSSION

Compound **1** (*trans*-phytol, Goodman *et al.*, 1973; Sims *et al.*, 1976), compound **3** (β-amyrin acetate, Manheim *et al.*, 1978), compound **6** (oleanolic acid, Mahato *et al.*, 1994; Ahmad *et al.*, 1994), compound **7** (kaempferol, Singh *et al.*, 2002), and compound **8** (kaempferol-3-*O*-rutinoside, Lee *et al.*, 1992) were characterized by comparing their physical and spectroscopic data with those of reported literatures.

Compound **2** was obtained as a colorless oil. The EI-MS spectrum of **2** showed a molecular ion peak at *m/z* 220 ($C_{15}H_{24}O$) and a base peak at m/z 109. The IR spectrum showed the presence of an OH group at 3415 cm^{-1}. The ^{1}H-NMR spectrum showed two secondary methyl groups at δ 0.81 (3H, d, *J* = 7.0 Hz) and 0.89 (3H, d, *J* = 7.0 Hz), an oxygenated methylene proton at δ 3.78 (1H, m), two olefinic protons at δ 5.40 (1H, dd, *J* = 16.0, 10.5 Hz), 5.97 (1H, d, *J* = 16.0 Hz) and four exomethylene protons at δ 4.84 (1H, s), 4.95 (1H, s), 5.01 (1H, s), 5.27 (1H, s). The ^{13}C-NMR spectrum exhibited the presence of 15 carbon signals, consisting of six olefinic carbon signals at δ 111.2, 113.6, 130.3, 138.6, 147.4, and 154.1, one oxygenated carbon signal at δ 76.7, and eight aliphatic signals at δ 21.2-53.2. These spectral data suggested that **2** was a sesquiterpene with a secondary alcohol, two exomethylene and a *trans* double bond. Based on the above mentioned data and the reported chemical structures of sesquiterpenes (Bohlmann *et al.*, 1982), the structure of **2** was determined to be 1-hydroxygermacra-4(15),5, 10(14)-triene. The NMR spectral and physical data of the compound **2** were in good agreement with those reported in the previous paper (Bohlmann *et al.*, 1982; Nagashima *et al.*, 1990).

Compound **4** was obtained as a colorless oil. The EI-MS spectrum of **4** showed a molecular ion peak at *m/z* 220 ($C_{15}H_{24}O$). The ^{1}H- and ^{13}C-NMR data of **4** were

almost same with **2**. The optical rotation of **4** ($[\alpha]_D$ 40.21°), which was opposite to that of **2** ($[\alpha]_D$ +70.51°, was same with value of *ent*-germacra-4(15),5,10(14)-trien-1β-ol (Nagashima *et al.*, 1990). The NMR spectral and physical data of the compound **4** were in good agreement with those reported in the previous paper (Bohlmann *et al.*, 1982), thus, the structure of **4** was determined to *ent*-germacra-4(15),5,10(14)-trien-1β-ol.

Compound **5** was obtained as a colorless oil and showed a molecular ion peak at m/z 220 ($C_{15}H_{24}O$) in EI-MS spectrum. The IR spectrum showed the presence of an OH group at 3500 cm^{-1}. The ^{1}H-NMR spectrum showed two methyl groups at δ 0.70 (3H, s) and 1.69 (3H, s), a carbinol proton at δ 3.11 (1H, dd, *J* = 11.5, 4.5 Hz) and four exomethylene protons at δ 4.54 (1H, s), 4.75 (1H, brs), 4.84 (1H, s), 4.86 (1H, s). The ^{13}C-NMR spectrum of **5** exhibited the presence of 15 carbon signals, consisting of four olefinic carbon signals at δ 106.8, 108.1, 148.5, and 150.3, one oxygenated carbon signal at δ 79.0. These spectral data suggested that **5** was a eudesmane sesquiterpene with a secondary alchol, two exomethylene groups. On the basis of the above mentioned data and literature survey on sesquiterpene (Bohlmann *et al.*, 1982), structure of compound **5** was determined as β-dictyopterol. The NMR spectral and physical data of compound **5** were in good agreement with those reported in the previous paper (Hu *et al.*, 1997).

Compound **9** was obtained as yellowish gum ($[\alpha]_D$ -194.31°). The FAB-MS spectrum gave a molecular formula ion peak ($[M+H]^+$) at *m/z* 531 ($C_{26}H_{27}O_{12}$). The ^{1}H-NMR spectrum showed signals by two *trans*-caffeoyl groups [δ 7.48/7.42 (1H each, d, *J* = 16.0 Hz), 7.04/7.04 (1H each, d, *J* = 2.5 Hz), 6.99/6.99 (1H each, d, *J* = 8.0 Hz), 6.77/6.77 (1H each, d, *J* = 8.0 Hz) and 6.23/6.14 (1H each, d, *J* = 16.0 Hz)] and three oxygenated protons [δ 3.98 (1H, dd, *J* = 9.5, 3.0 Hz), 5.29 (1H, dt, *J* = 7.0, 3.5 Hz), and 5.38 (1H, br.dd, *J* = 9.5, 4.5 Hz)]. The ^{13}C-NMR spectrum

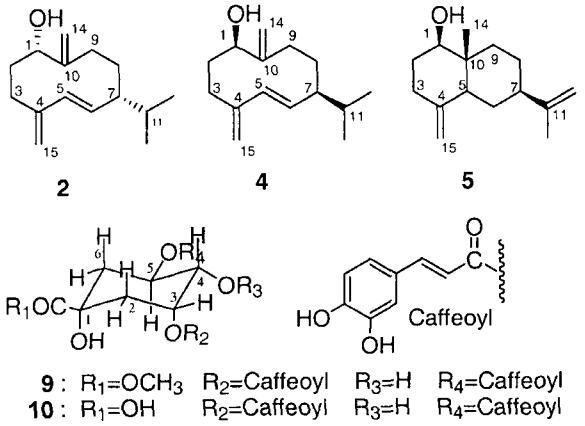

Fig. 1. Structures of compounds **2**, **4**, **5**, **9**, and **10**

Table I. Cytotoxicity of Compounds **1~10**

	EC_{50} values*(μM)				
Compounds	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
4	14.21	17.06	12.30	14.34	14.17
5	18.57	17.97	5.91	1.52	9.11
10	>30.0	9.92	9.81	>30.0	>30.0
1~3 & 6~9	>30.0	>30.0	>30.0	>30.0	>30.0

*EC_{50} value of compound against each cancer cell line, which was defined as a concentration (μM) that caused 50% inhibition of cell groeth *in vitro*.

showed two methylene carbons at δ 35.6 and 35.9, four oxygenated carbons at δ 67.9, 71.1, 72.1, and 73.8, and a carbonyl carbon signal at δ 174.7. The above ^{1}H- and ^{13}C-NMR spectral data were typical in dicaffeoyl quinic acid derivatives (Clifford *et al.*, 1986). The position of two caffeoyl groups was established by the downfield shift of the H-3 (δ 5.38) and H-5 (δ 5.29) in the ^{1}H-NMR spectrum and C-3 (δ 72.1) and C-5 (δ 71.1) in the ^{13}C-NMR spectrum. Thus, the structure of compound **9** was determined as methyl 3,5-dicaffeoyl quinate. The NMR spectral and physical data of compound **9** were in good agreement with those reported in the previous paper (Lin *et al.*, 1999).

Compound **10** was obtained as yellowish gum ($[\alpha]_D$ -230.14°). The ^{1}H- and ^{13}C-NMR spectra of **10** were quite similar to those of **9**. The major difference in the NMR spectra was the absence of methoxy signal (δ 53.0 in **9**) and the presence of acid group (δ 178.8, in **10**). Thus, the structure of compound **10** was determined as 3,5-dicaffeoyl quinic acid. The NMR spectral and physical data of compound **10** were in good agreement with those reported in the previous paper (Basnet *et al.*, 1996).

Compounds **2-10** were first isolated from this plant. The cytotoxicities of the compounds were tested by SRB (Sulforhodamin B) bioassay against five cultured human tumor cells. Of them, compound **4** showed moderate cytotoxicity against five tumor cell lines with ED_{50} values of 12.30~17.06 μg/mL, the compound **5** potent cytotoxicity with ED_{50} values of 1.52~18.57 μg/mL ant the compound **10** moderate cytotoxicity with ED_{50} values of 9.81~30.0 μg/mL. The other compounds were not active against tested five human tumor cell lines (Table I).

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