Natural Product Sciences
10(6) : 335-340 (2004)

Phytochemical Constituents of the Aerial Parts from *Aster hispidus*

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Abstract – The chromatographic separation of the MeOH extract of the aerial parts from *Aster hispidus* (Compositae) led to the isolation of eight compounds. Their structures were established by spectroscopic methods to be β-amyrin (**1**), oleanolic acid (**2**), (2*R*)-1, 2-*O*-(9Z, 12Z, 15Z-dioctadecatrienoyl)-3-*O*-β-D-galactopyranosyl glycerol (**3**), *trans*-phytol (**4**), 9, 12, 15-octadecatrienoic acid (**5**), kaempferol (**6**), 3,5-dicaffeoyl quinic acid (**7**), 3,4-dicaffeoyl quinic acid (**8**) and kaempferol-3-*O*-rutinoside (**9**). Compounds **1**, **3~6** and **9** showed non-specific moderate cytotoxicity against five human tumor cell lines (5.44~23.51 μg/ml). The other compounds were of marginal activity against tested five human cancer cell lines (9.05~>30.0 μg/ml).
Keywords – *Aster hispidus*, Compositae, terpenoid, flavonoid, cytotoxicity

Introduction

Aster hispidus (Compositae), a perennial herb, is distributed mainly in the coast of South Korea, and its aerial parts have been used to treat diuresis in Korean traditional medicine. But, the phytochemical studies on this plant has not been found in the literatures. As part of our systematic study of Korean Compositae plants, we have investigated the constituents of *A. hispidus*. The chromatographic separation of the MeOH extract of the aerial parts of *A. hispidus* led to the isolation of nine compounds. Their structures were characterized by spectral means to be β-amyrin (**1**), oleanolic acid (**2**), (2*R*)-1, 2-*O*-(9Z, 12Z, 15Z-dioctadecatrienoyl)-3-*O*-β-D-galactopyranosyl glycerol (**3**), *trans*-phytol (**4**), 9, 12, 15-octadecatrienoic acid (**5**), kaempferol (**6**), 3,5-dicaffeoyl quinic acid (**7**), 3,4-dicaffeoyl quinic acid (**8**) and kaempferol-3-*O*-rutinoside (**9**). This paper describes the isolation, structural characterization and cytotoxic activities of the compounds.

Experimental

Instruments and reagents – 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, 70230 mesh and 230400 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 – *Aster hispidus* (Compositae) was collected in Jeju, Korea in August 2001. The voucher specimen (SKK-01-021) was deposited at the Herbarium of College of Pharmacy, 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 adenocarcinoma), SK-OV-3 (ovarian), SK-MEL-2 (skin melanoma), XF498 (CNS) and HCT15 (colon) (Skehan *et al.*, 1990).

Extraction and isolation – The dried and chopped aerial parts of *Aster hispidus* (4.5 kg) were extracted with MeOH three times at room temperature. The resultant MeOH extract (420 g) followed by successive solvent partition gave hexane (30 g), CH_2Cl_2 (13 g), EtOAc (12 g) and BuOH (35 g) soluble fractions. The hexane soluble fraction (30 g) was chromatographed over silica gel column using the gradient solvent system of hexane:EtOAc (10:1~1:1) and CH_2Cl_2:MeOH (20:1) to give five subfractions (H1~H5). The subfraction H2 (5.5 g) was chromatographed with silica gel column eluting with hexane:EtOAc (5:1) to give four subfractions (H21~H24). The subfraction H23 (700 mg) was chromatographed on a RP-18 Lobar®-A column (100% MeCN) and purified

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over silica gel column (CH_2Cl_2) and HPLC (hexane: EtOAc = 5:1) to afford **1** (15 mg).

The subfraction H3 (3.7 g) was chromatographed with Sephadex LH-20 column chromatography (CH_2Cl_2: MeOH = 1:1) to give three subfractions (H31~H33). The subfraction H32 (1 g) was further purified with Sephadex LH-20 (CH_2Cl_2:MeOH = 1:1) and silica gel column chromatography (CH_2Cl_2: MeOH = 40:1) to afford **2** (70 mg).

The subfraction H5 (3 g) was chromatographed over silica gel column (CH_2Cl_2:MeOH = 20:1) to give five subfractions (H51~H53). The subfraction H53 (1 g) was further subjected to Sephadex LH-20 (CH_2Cl_2:MeOH = 1:1) and silica gel column chromatography ($CHCl_3$: MeOH = 20:1) to give three subfractions (H531~H533). The subfraction H532 (670 mg) was purified with RP-18 Lobar®-A (100% MeOH) and silica gel column (hexane : EtOAc = 1:1) to afford **3** (10 mg).

The CH_2Cl_2 soluble fraction (13 g) was chromatographed through silica gel column using the gradient solvent system of hexane:EtOAc (3:1 and 1:1) and CH_2Cl_2: MeOH (20:1 and 5:1) to give five subfractions (AM1~AM5). The subfraction AM1 (190 mg) was purified over a silica gel column (hexane:EtOAc = 7:1), Sephadex LH-20 column (CH_2Cl_2:MeOH = 1:1) and HPLC (hexane:EtOAc = 7:1) to afford **4** (70 mg). The subfraction AM2 (500 mg) was purified with Sephadex LH-20 (CH_2Cl_2:MeOH = 1:1) and over silica gel column chromatography (hexane:EtOAc = 3:1) to afford **5** (150 mg).

The EtOAc soluble fraction (12 g) was chromatographed over silica column using a solvent system of ($CHCl_3$: EtOAc:MeOH = 3:2:1) to give five subfractions (AE1AE5). The subfraction AE3 (2 g) was further subjected to Sephadex LH-20 (CH_2Cl_2:MeOH = 1:1) column chromatography to give three subfractions (AE31~AM33). The subfraction AE33 (200 mg) was purified over silica gel column ($CHCl_3$:EtOAc:MeOH = 1:1:1), Sephadex LH-20 (CH_2Cl_2:MeOH = 1:1) and with silica gel column chromatography ($CHCl_3$:EtOAc:MeOH = 3:2:1) to afford **6** (12 mg) and **7** (10 mg). The subfraction AE4 (900 mg) was further purified with Sephadex LH-20 (CH_2Cl_2: MeOH = 1:1) and silica gel column chromatography ($CHCl_3$: EtOAc:MeOH = 1:1:1) to afford **8** (12 mg). The subfraction AE5 (3.3g) was chromatographed over silica gel column eluted with EtOAc:MeOH:H_2O (9:2:0.5), and Sephadex LH-20 (MeOH to afford **9** (15mg).

Compound 1 – White powder; EIMS *m/z* (rel. int.,%): 426 (M^+, 27), 411 (8), 393 (8), 218 (100), 207 (10), 203 (32), 189 (22), 135 (26), 109 (35), 107 (23); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.79, 0.83 (each 3H, s, H-24, 28), 0.89 (6H, s, H-29, 30), 0.94, 0.97, 1.01 and 1.15 (each 3H, s, H-25, 23, 26, 27), 3.23 (1H, m, H-3α) and 5.14 (1H, t, *J* = 4.0 Hz, H-12); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 15.6 (C-24), 15.7 (C-25), 17.0 (C-26), 18.5 (C-6), 23.7 (C-11, 30), 26.2 (C-15, 27), 27.0 (C-16), 27.2 (C-2), 28.1 (C-23), 28.4 (C-28), 31.0 (C-20), 32.5 (C-17), 32.9 (C-7), 33.3 (C-29), 34.7 (C-21), 37.1 (C-10), 37.2 (C-22), 38.6 (C-1), 38.9 (C-4, 8), 41.8 (C-14), 46.7 (C-19), 47.2 (C-18), 47.7 (C-9), 55.3 (C-5), 79.3 (C-3), 121.6 (C-12), 145.3 (C-13).

Compound 2 – White powder; mp 197°, EIMS *m/z* (rel. int.,%) : 456 (M^+, 6), 248 (100), 207 (30), 204 (32), 203 (72), 189 (30); ^{1}H-NMR (500MHz, $CDCl_3$) : δ 0.74, 0.79, 0.89, 0.91, 0.92, 0.98 and 1.12 (each 3H, s), 2.83 (1H, br. dd, *J*= 4.0, 14.0 Hz), 3.22 (1H, br. dd, *J* = 4.0, 9.5 Hz) and 5.28 (each 1H, m); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 16.0 (C-25), 16.5 (C-24), 17.8 (C-26), 18.9 (C-6), 23.8 (C-30, 16, 11), 26.2 (C-27), 28.2 (C-2), 28.3 (C-15), 28.7 (C-23), 31.0 (C-20), 33.3 (C-7, 22 and 29), 34.3 (C-21), 37.4 (C-10), 38.9 (C-1), 39.4 (C-4), 39.8 (C-8), 42.0 (C-18,14), 46.7 (C-19, 17), 48.3 (C-9), 55.9 (C-5), 79.7 (C-3), 123.3 (C-12), 144.3 (C-13), 184.3 (C-28).

Compound 3 – Colorless oil; ^{1}H-NMR (500 MHz, $CDCl_3$): 5.36 (12H, m, H-9", 9"', 10", 10"', 12", 12"', 13", 13"', 15", 15"', 16" and 16"'), 5.30 (1H, m, H-2), 4.39 (1H, dd, *J* = 3.5, 12.0 Hz, H-1a), 4.28 (1H, d, *J* = 7.5 Hz, H-1'), 4.21 (1H, dd, *J* = 6.5, 12.0 Hz, H-1b), 4.02 (1H, d, *J* = 3.0 Hz, H-4'), 3.99 (1H, dd, *J* = 6.0, 12.0 Hz, H-6'a), 3.91 (1H, dd, *J* = 5.0, 11.0 Hz, H-3a), 3.89 (1H, dd, *J* = 3.5, 12.0 Hz, H-6'b), 3.75 (1H, dd, *J* = 6.0, 11.0 Hz, H-3b), 3.65 (1H, dd, *J* = 7.5, 9.5 Hz, H-2'), 3.60 (1H, dd, *J* = 3.0, 9.5 Hz, H-3'), 3.55 (1H, br. dd, *J* = 5.0 Hz, H-5'), 2.80 (8H, m, H-11", 11"', 14", 14"'), 2.32 (4H, dd, *J* = 8.0, 15.5 Hz, H-2", 2"'), 2.06 (8H, m, H-8", 8"', 17", 17"'), 1.61 (4H, m, H-3", 3"'), 1.30 (16H, m, H-4", 4"', 5", 5"', 6", 6"', 7", 7"'), 0.97 (6H, t, *J* = 7.5 Hz, H-18", 18"'); ^{13}C-NMR (125 MHz, $CDCl_3$) : δ 174.0, 173.7 (C-1", 1"'), 132.2, 130.5, 130.5, 128.5, 128.5, 128.0, 127.9, 127.3 (C-9", 9"', 10", 10"' 12", 12"', 13", 13"', 15", 15"', 16" and 16"'), 104.0 (C-1'), 74.7 (C-5'), 73.7 (C-3'), 72.0 (C-2'), 70.4(C-2), 69.8 (C-4'), 68.7 (C-3), 63.2 (C-1), 62.9 (C-6'), 34.5, 34.4 (C-2", 2"'), 29.8, 29.4, 29.4, 29.3, 29.3 (C-4,7" and C-4"',7"'), 27.5 (C-8", 8"'), 25.9 (C-11", 11"', 14" and 14"'), 25.1, 25.1 (C-3", 3"'), 20.8 (C-17", 17"'), 14.6 (C-18", 18"').

Compound 4 – Colorless oil; $[\alpha]_D$ +0.2° (*c* 0.3, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3443 (OH), 1667 $(C=C)^{-1}$; EIMS *m/z*: 296 $[M]^+$; ^{1}H-NMR, (500 MHz, $CDCl_3$): 5.42 (1H, tq, *J* = 1.0, 7.0 Hz, H-2), 4.16 (2H, d, *J* = 7.0 Hz, H-

1), 2.00 (2H, m, H-4), 1.68 (3H, s, CH_3-3a), 1.01~1.62 (19H, m, CH_2-5, 6, 8, 9, 10, 12, 13, 14, 15, CH-7, 11, 15), 0.85~0.88 (12H, m, CH_3-7a, 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, 20.0, 16.4.

Compound 5 – Colorless gum; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.36 (6H, m, H-9, 10, 12, 13, 15, 16), 2.80 (4H, br. t, *J* = 5.5 Hz, H-11, 14), 2.34 (2H, t, *J* = 7.5 Hz, H-2), 2.06 (4H, m, H-8, 17), 1.62 (2H, m, H-3), 1.31 (8H, m, $-CH_2\times 4$), 0.97 (3H, t, *J* = 7.3 Hz, Me-18); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 180.6 (C-1), 132.2, 130.5, 128.5, 128.5, 128.0, 127.4 (C-9, 10, 12, 13, 15, 16), 34.4 (C-2), 19.9, 29.4, 29.3, 29.2 (C-4, 5, 6, 7), 27.4 (C-8), 25.9, 25.8 (C-11, 14), 24.9 (C-3), 20.8 (C-17), 14.6 (C-18).

Compound 6 – Yellow powder; mp 306°; EIMS *m/z* (rel. int.,%): 286 (M^+, 3), 256 (8), 128 (100), 118 (33), 113 (67), 97 (95); ^{1}H-NMR (500 MHz, MeOD): δ 5.14 (1H, d, *J* = 2.0 Hz, H-8), 5.95 (1H, d, *J* = 2.0 Hz, H-6), 6.73 (2H, d, *J* = 8.5 Hz, H-3', 4'), 7.10 (2H, d, *J* = 8.5 Hz, H-2', 6'); ^{13}C-NMR (125 MHz, MeOD): δ 144.2 (C-2), 136.9 (C-3), 170.8 (C-4), 164.2 (C-5), 99.7 (C-6), 168.1 (C-7), 92.3 (C-8), 149.3 (C-9), 107.7 (C-10), 125.5 (C-1'), 130.8 (C-2'), 116.3 (C-3'), 158.6 (C-4'), 116.1 (C-5'), 131.1 (C-6').

Compound 7 – Yellow gum; $[\alpha]_D$ −220.4° (*c* 0.2, MeOH); FABMS *m/z*: 517 $[M+H]^+$; ^{1}H-NMR (500 MHz, MeOD): δ 2.13 (br. d, *J* = 12.5 Hz), 1.86 (br. m), 5.49 (dd. *J* = 10.0, 4.5 Hz), 3.39 (dd, *J* = 10.0, 3.0 Hz), 5.39 (br. dd, *J* = 3.5, 6.5 Hz), 1.91 (br. m), 6.25/6.22 (d, *J* = 15.5 Hz), 7.46/7.45 (d, *J* = 15.5 Hz), 7.06/7.06 (s), 6.76/6.75 (d, *J* = 8.0 Hz), 6.96/6.96 (d, *J* = 8.0 Hz); ^{13}C-NMR (125 MHz, MeOD): δ 75.5 (C-1), 37.1 (C-2), 73.8 (C-3), 71.8 (C-4), 72.0 (C-5), 40.1 (C-6), 167.5(C-1"), 167.3 (C-1"), 117.0 (C-2'), 116.9 (C-2"), 145.8 (C-3'), 145.5 (C-3"), 126.8 (C-4'), 126.7 (C-4"), 115.9 (C-5') 115.7 (C-5"), 146.8 (C-6'), 146.8 (C-6"), 149.5 (C-7'), 149.4 (C-7"), 116.3 (C-8'), 116.3 (C-8"), 122.4 (C-9'), 122.0 (C-9"), 178.9 (COOH).

Compound 8 – Yellow gum; $[\alpha]_D$ −219.0° (*c* 0.2, MeOH); FABMS *m/z* : 517 $[M+H]^+$; ^{1}H-NMR (500 MHz, MeOD): δ 7.59 (1H, d, *J* = 16.0 Hz), 7.50 (1H, d, *J* = 16.0 Hz), 7.02 (1H, d, *J* = 2.0 Hz), 6.99 (1H, d, *J* = 2.0 Hz), 6.90 (1H, dd, *J* = 2.0, 8.0 Hz), 6.88 (1H, dd, *J* = 2.0, 8.0 Hz), 6.74 (1H, d, *J* = 8.0 Hz), 6.73 (1H, d, *J* = 8.0 Hz), 6.27 (1H, d, *J* = 16.0 Hz), 6.19 (1H, d, *J* = 16.0 Hz), 5.68 (1H, dt, *J* = 5.0, 10.0 Hz), 5.12 (1H, dd, *J* = 3.0, 10.0 Hz), 4.35 (1H, dt, *J* = 2.5, 3.0 Hz), 2.29 (1H, dd, *J* = 3.0, 14.0 Hz), 2.20 (2H, m), 2.02 (1H, dd, *J* = 6.0, 14.0 Hz); ^{13}C-NMR (125 MHz, MeOD): δ 77.9 (C-1), 40.2 (C-2), 70.1 (C-3), 77.5 (C-4), 71.0 (C-5), 39.5 (C-6), 169.3 (C-1"), 169.4 (C-1'), 115.7 (C-2'), 115.7 (C-2"), 148.2 (C-3'), 148.4 (C-3"), 128.4 (C-4'), 128.4 (C-4"), 115.9 (C-5') 115.8 (C-5"), 147.5 (C-6'), 147.5 (C-6"), 150.3 (C-7'), 150.3 (C-7"), 117.2 (C-8'), 117.4 (C-8"), 123.8 (C-9'), 123.8 (C-9"), 179.8 (COOH).

Compound 9 – Yellow powder; mp 170°; FABMS *m/z* : 617 $[M+H]^+$; ^{1}H-NMR (500 MHz, MeOD): δ 1.11 (3H, d, *J*=6.5Hz), 4.51 (1H, br. s, rha-1), 5.12 (1H, d, *J* = 7.5 Hz, glc-1), 6.21 (1H, d, *J* = 2.0Hz), 6.40 (1H, d, *J* = 2.0Hz), 6.88 (2H, dd, *J* = 2.0, 7.0 Hz), 8.05 (2H, dd, *J* = 2.0, 7.0 Hz); ^{13}C-NMR (125 MHz, MeOD): δ 18.0 (C-6'"), 94.8 (C-8), 99.9 (C-6), 102.2 (C-1'"), 104.6 (C-1"), 105.5 (C-10), 116.0 (C-3', 5'), 122.5 (C-1'), 132.2 (C-2', 6'), 135.4 (C-3), 158.4 (C-2), 159.2 (C-2), 161.2 (C-4), 162.7 (C-5), 165.7 (C-7), 179.0 (C-4).

Results and Discussion

Compound **1** was obtained as a white powder. The EIMS spectrum of **1** showed a molecular ion peak at *m/z* 426. The ^{1}H-NMR spectrum showed eight methyl groups at δ 0.79, 0.83 (each 3H, s, H-24, 28), 0.89 (6H, s, H-29, 30), 0.94, 0.97, 1.01 and 1.15 (each 3H, s, H-25, 23, 26, 27), an olefinic protons at δ 5.14 (1H, t, *J* = 4.0 Hz, H-12) and an oxygenated methine proton at δ 3.23 (1H, m, H-3α). The ^{13}C-NMR spectrum exhibited the presence of 30 carbon signals, consisting of eight methyl signals at δ 15.6 (C-24), 15.7 (C-25), 17.0 (C-26), 23.7 (C-30), 26.2 (C-27), 28.1 (C-23), 28.4 (C-28), 33.3 (C-29), two olefinic carbon signals at δ 121.6 (C-12), 145.3 (C-13), and an oxygenated carbon signal at δ 79.3 (C-3). These spectral data suggested that **1** was a triterpene. Based on the above mentioned data and the reported chemical structures of triterpenes (Mahato & Kundu, 1994), the structure of **1** was determined to be β-amyrin. The NMR spectral and physical data of the compound **1** were in good agreement with those reported in the previous paper (Lee *et al.*, 2003).

Compound **2** was obtained as a white powder. The EIMS spectrum of **2** showed a molecular ion peak at *m/z* 456. The ^{1}H- and ^{13}C-NMR spectral data of compound **2** were almost same with those of compound **1**. The only difference in the ^{13}C-NMR spectrum was the presence of an acid signal at δ 184.3 (C-28) in **2**. Based on the above mentioned data and the reported chemical structures of triterpenes (Mahato & Kundu, 1994), the structure of **2** was determined to be oleanolic acid. The NMR spectral and physical data of the compound **2** were in good agreement with those reported in the previous paper

(Ahmad & Rahman, 1994).

Compound **3** was obtained as colorless oil. The spectral data of **3** showed the presence of a sugar and an aliphatic long chain with double bonds, indicating of a glycolipid (Dey & Harborne, 1990). The ^{1}H-NMR signals at 4.28 (d, J = 7.5 Hz), 4.02 (d, J = 3.0 Hz), 3.99 (dd, J = 6.0, 12.0 Hz), 3.89 (dd, J = 3.5, 12.0 Hz), 3.65 (dd, J = 7.5, 9.5 Hz), 3.60 (dd, J = 3.0, 9.5 Hz) and 3.55 (br. dd, J = 5.0 Hz) indicated the presence of a β-D-galactopyranose (Jung & kang, 1996; Kobayashi *et al.*, 1992). An ABMXY coupling system connected to oxygenated carbons (63.2, 68.7 and 70.4) observed in the ^{1}H-NMR spectra suggested a glycerol moiety (Jung & Kang, 1996; Kobayashi *et al.*, 1992). In ^{13}C-NMR spectrum, two carbonyl signals at 173.7 and 174.0 suggested two acyl group moieties. The geometry of double bonds of acyl group moieties was determined to be *cis*-form based on the ^{13}C-NMR chemical shift at 25.9 (C-11", 11"', 14" and 14"') and the carbon signal of *trans*-form of double bond shows at 32~33 (Jung and Kang, 1996). Based on the above consideration and the comparison of the data in the previous papars (Jung & kang, 1996; Kobayashi *et al.*, 1992), the structure of **3** was established as (2*R*)-1, 2-*O*-(9Z, 12Z, 15Z-dioctadecatrienoyl)-3-*O*-β-D-galactopyranosyl glycerol.

Compound **4** was obtained as colorless oil. From the EIMS, ^{1}H- and ^{13}C-NMR spectral data, the molecular formula was deduced to be $C_{20}H_{40}O$. The ^{1}H- and ^{13}C-NMR spectra showed the typical pattern of linear diterpene. Based on the above consideration and the comparison of the data in the previous papars (Goodman *et al.*, 1973; Sims & Pettus, 1976), the structure of **4** was established as *trans*-phytol.

The compound **5** was identified to be 9, 12, 15-octadecatrienoic acid by ^{1}H- and ^{13}C-NMR data and GCMS analysis. The NMR spectral and physical data of the compound **5** were in good agreement with those reported in the previous paper (Lee *et al.*, 2002).

Compound **6** was obtained as a yellow powder. The EIMS spectrum of **6** showed a molecular ion peak at *m/z* 286. The ^{1}H-NMR spectrum showed the AB system at 6.73 (2H, d, J = 8.5 Hz) and 7.10 (2H, d, J = 8.5 Hz), and also showed two *meta*-coupled doublets at 5.14 (1H, d, J = 2.0 Hz) and 5.95 (1H, d, J = 2.0 Hz). The ^{13}C-NMR spectrum exhibited 15 carbon signals, consisting of fourteen olefinic signals at δ 92.3~168.1, and a carbonyl carbon signal at δ 170.8. These spectral data suggested that **6** was a flavonol derivative. Based on the above mentioned data and the reported chemical structures of flavonoids (Lee *et al.*, 2003), the structure of **6** was determined to be kaempferol. The NMR spectral and physical data of the compound **6** were in good agreement with those reported in the previous paper (Markham *et al.*, 1978).

Compound **7** was obtained as yellowish gum ($[\alpha]_D$ -220.4°) and its molecular formula was determined to be $C_{25}H_{24}O_{12}$ by FABMS (*m/z* 517, $[M+H]^+$), ^{1}H- and ^{13}C-NMR spectra data. Its IR spectrum displayed absorption bands at 3300 and 1690 cm^{-1}, indicating the presence of hydroxy and ester groups. The ^{1}H- and ^{13}C-NMR spectral data of **7** indicated the presence of two *trans*-caffeoyl groups [δ 7.45/7.46 (1H each, d, J = 15.5 Hz), 7.06/7.06 (1H each, s), 6.96/6.96 (1H each, dd, J = 8.0 Hz), 6.75/6.76 (1H each, d, J = 8.0 Hz) and 6.22/6.25 (1H each, d, J=15.5 Hz)] and three oxygenated protons [δ 3.39 (1H, dd, J = 3.0, 10.0 Hz), 5.39 (1H, dd, J = 3.5, 6.5 Hz), and 5.49 (1H, dd, J = 4.5, 10.0 Hz)]. The ^{13}C-NMR spectrum showed two methylene carbons at δ 37.1 and 40.1, four oxygenated carbons at δ 71.8, 72.0, 73.8 and 75.5, and a carbonyl carbon signal at δ 178.9. The ^{1}H- and ^{13}C-NMR spectral data were typical of dicaffeoyl quinic acid derivatives (Clifford, 1986). The position of two caffeoyl groups was established by the downfield shift of the H-3 (δ 5.39) and H-5 (δ 5.49) in the ^{1}H-NMR spectrum and of the C-3 (δ 73.8) and C-5 (δ 72.0) in the ^{13}C-NMR spectrum. Thus, the structure of compound **7** was determined as 3,5-dicaffeoyl quinic acid. The NMR spectral and physical data of compound **7** were in good agreement with those reported in the previous paper (Basnet *et al.*, 1996).

Compound **8** was obtained as yellowish gum ($[\alpha]_D$ 219.0°) and its molecular formula was determined to be $C_{25}H_{23}O_{12}$ by FABMS (*m/z* 517, $[M+H]^+$), ^{1}H- and ^{13}C-NMR data. The ^{1}H- and ^{13}C-NMR spectra of **8** were also similar to those of **7**. The major differences were the chemical shift of H-4 and H-5 at the quinic acid moiety in **8**. The H-4 of **8** was shifted downfield by 1.73 ppm and the H-5 shifted upfield by 1.04 ppm relative to those of **7**. Also, the H-3 and H-4 was shifted downfield by about 1.6 ppm relative those of free quinic acid (Iwahashi *et al.*, 1985). Thus, the structure of compound **8** was determined as 3,4-dicaffeoyl quinic acid. The NMR spectral and physical data of compound **8** were in good agreement with those reported in the previous paper (Basnet *et al.*, 1996).

Compound **9** was obtained as a yellow powder. The FABMS spectrum of **9** showed a molecular ion peak at *m/z* 617 ($[M+H]^+$). The ^{1}H- and ^{13}C-NMR spectra of compound **9** were almost same as those of compound **6**. The only difference was the sugar moiety. The anomeric signals were observed in the ^{1}H-NMR spectrum [δ 4.51

(1H, br. s, rha-1) and 5.12 (1H, d, J = 7.5 Hz, glc-1)] and ^{13}C-NMR spectrum [δ 102.2 (C-1‴) and 104.6 (C-1″)] in **9**. These spectral data suggested that **9** was a flavonol glycoside. Based on the above mentioned data and the reported chemical structures of flavonoids (Lee *et al.*, 2003), the structure of **9** was determined to be kaempferol-3-*O*-rutinoside. The NMR spectral and physical data of the compound **9** were in good agreement with those reported in the previous paper (Han *et al.*, 2004).

Compounds (**1~9**) were tested for their cytotoxicity

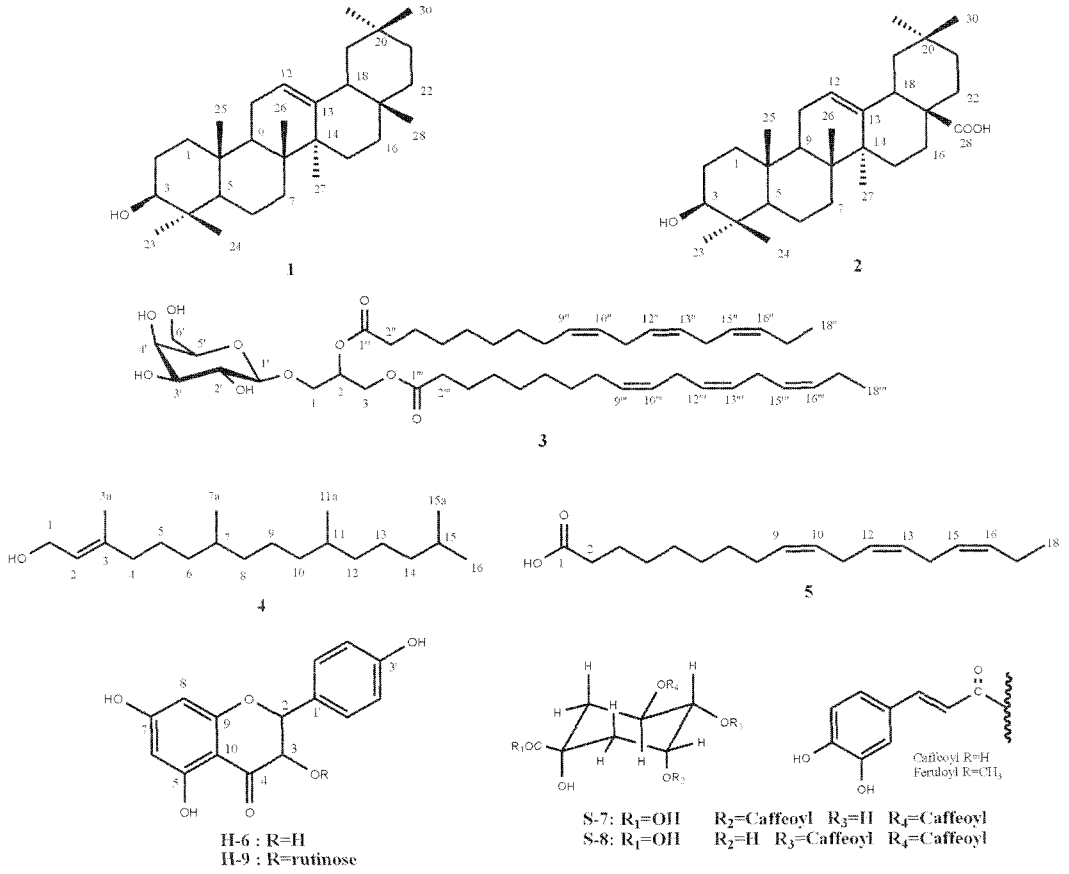

Fig. 1. Structures of compounds 1~9.

Table 1. Cytotoxicity of compounds 1~9

Compounds \Cancer Cell Lines	ED_{50} values [a]				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT1
1	14.26	9.10	5.49	12.31	5.44
2	>30.0	9.05	>30.0	>30.0	>30.0
3	14.53	12.91	9.97	15.09	18.54
4	11.47	5.50	7.84	8.26	4.47
5	14.22	19.17	10.08	19.47	16.24
6	8.43	8.70	10.65	13.47	23.51
7	>30.0	25.19	23.41	>30.0	>30.0
8	>30.0	32.19	25.48	>30.0	>30.0
9	7.32	10.63	8.25	12.78	14.84
Doxorubicin	0.018	0.071	0.009	0.008	0.381

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

against human tumor cell lines (Table). Compounds **1**, **3~6** and **9** showed non-specific moderate cytotoxicity against five human tumor cell lines (5.44~23.51 μg/ml). The other compounds were little activity against tested five human cancer cell lines (9.05~ >30.0 μg/ml).

Acknowledgments

This research was supported in Korea Science and Engineering Foundation (R05-2004-000-10015-0). The authors would like to thank Hur, Yeon and Yi, Hyung Ju at Korea Basic Science Instituent for the measurements of EI-MS and FAB-MS spectra.

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(Accepted December 6, 2004)