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Phytochemical Constituents of *Amomum xanthioides*

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Abstract – Seven monoterpenes, three sesquiterpenes, three phenolics and one flavonoid were isolated from the MeOH extract of *Amomum xanthioides*. Their structures were determined by spectroscopic methods to be caryophyllene oxide (**1**), bornyl acetate (**2**), nerolidol (**3**), spathulenol (**4**), (−)-borneol (**5**), (+)-5-endo-hydroxycamphor (**6**), vanillic acid (**7**), protocatechuic acid methyl ester (**8**), betulabuside A (**9**), (1*R*,4*S*,6*R*)-6-hydroxyfenchan-2-one-6-*O*-β-D-glucopyranoside (**10**), (1*S*,4*R*,6*S*)-6-hydroxybornan-2-one-6-*O*-β-D-glucopyranoside (**11**), (1*R*,2*S*,4*S*,5*R*)-angelicoidenol 2-*O*-β-D-glucopyranoside (**12**), 1-*O*-vanilloyl-β-D-glucopyranoside (**13**), and quercetin-3-rhamnopyranoside (**14**). Compounds **6**-**14** were isolated for the first time from this plant source. Compounds **3** and **4** exhibited moderate cytotoxicity against four human cancer cell lines *in vitro* using a SRB bioassay.

Key words – *Amomum xanthioides*, Zingiberaceae, Monoterpenes, Sesquiterpenes, Cytotoxicity

Introduction

The seeds of *Amomum xanthioides* (Zingiberaceae) have been used in traditional medicine for the treatment of stomach and digestion disorder (Kitajima *et al*., 2003). The extract of this species exhibited antidiabetic (Park *et al*., 2001), and antimicrobial activities (George *et al*., 2006). The seed of *A. xanthioides* is listed in Japanese Pharmacopoeia as “amomum seed” and contains essential oil (1 - 1.5%) rich in monoterpenoids (borneol, linalool, camphene and nerolidol) (Zhang *et al*., 1989). As parts of our continuing search for biological active compounds from natural sources, we investigated the constituents of *Amomum xanthioides*. Thus, we isolated seven monoterpenes, three sesquiterpenes, three phenolics and one flavonoid from its MeOH extract. The isolated compounds were tested for their cytotoxic activities against four human cancer cell lines *in vitro* using a SRB bioassay.

Experimental

General – Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1020 Polarimeter. IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. UV spectra were recorded with a Schimadzu UV-1601 UV-Visible spectrophotometer. NMR spectra were recorded on a Varian UNITY INOVA 500 NMR spectrometer. ESIMS and FABMS data were obtained on a JEOL JMS700 mass spectrometer, and LC-ESI/MS data on an Agilent 1100LC/ MSD trap SL LC/MS. Preparative HPLC was performed using a Gilson 306 pump with a Shodex refractive index detector and Alltech Silica 5 μ column (250 × 22 mm) or Econosil® RP-18 10 μ column (250 × 22 mm). Silica gel 60 (Merck, 70~230 mesh and 230~400 mesh) was used for column chromatography. TLC was performed using Merck precoated Silica gel F_{254} plates and RP-18 F_{254s} plates. The packing material for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia Co.). Low-pressure liquid chromatography was performed over Merck LiChroprep Lobar®-A Si 60 (240 × 10 mm) or LiChroprep Lobar®-A RP-18 (240 × 10 mm) columns with a FMI QSY-0 pump (ISCO).

Plant materials – The seeds of *Amomum xanthioides* (2.5 kg) were bought at kyungdong market, which were imported from China. A voucher specimen (SKKU-2007-12B) of the plant was deposited at the College of Pharmacy in Sungkyunkwan University.

Extraction and isolation – The seeds of *A. xanthioides* (2.5 kg) were extracted at room temperature with 80% MeOH and evaporated under reduced pressure to give a residue (210 g), which was dissolved in water (800 mL) and partitioned with solvent to give hexane (18 g), $CHCl_3$

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(11 g), and BuOH (23 g) soluble portions. The hexane fraction (18 g) was separated over a silica gel column (hexane : EtOAc = 10 : 1 – 1 : 1) to yield seven fractions (H1-H7). Fraction H2 (1.4 g) was further seperated over a RP-C_{18} silica gel column (85% MeOH) and purified over a silica gel prep. HPLC (hexane : EtOAc = 50 : 1, over 30 min at a flow rate of 2.0 mL/min, Alltech Silica 5 μ column 250 × 10 mm, Shodex RI-101 refractive index detector) to yield compounds **1** (6 mg) and **2** (22 mg). Fraction H3 (1.3 g) was purified with Sephadex LH-20 column (CH_2Cl_2 : MeOH = 1 : 1) and a silica gel prep. HPLC (hexane : EtOAc = 18 : 1, Alltech Silica 5 μ column 250 × 10 mm) to yield compound **3** (80 mg). In turn, fraction H4 (2.0 g) and fraction H6 (1.8 g) were seperated over RP-C_{18} silica gel column and purified with a silica Lobar A®-column to give compounds **4** (12 mg) and **5** (9 mg), respectively. The $CHCl_3$ fraction (11 g) was separated over a silica gel column with a solvent system of $CHCl_3$: MeOH (15 : 1 – 1 : 1) as the eluant to give six fractions (C1-C6). The fraction C2 (0.9 g) was purified with a silica gel prep. HPLC ($CHCl_3$: MeOH = 100 : 1) to afford compound **6** (19 mg). Fraction C3 (1.5 g) was separated with RP-C_{18} silica gel column with 45% MeOH and purified with a silica gel prep. HPLC ($CHCl_3$: MeOH = 10 : 1) to afford compound **7** (6 mg). Fraction C4 (0.5 g) was purified with a silica gel prep. HPLC (hexane : $CHCl_3$: MeOH = 3 : 5 : 0.5) to afford compound **8** (7 mg). Similarly, the BuOH fraction (23 g) was separated over a silica gel column with a solvent system of $CHCl_3$: MeOH : H_2O (4 : 1 : 0.1 – 5 : 2 : 0.2) as the eluant to give five fractions (B1-B5). Fraction B2 (2.4 g) was separated by Sephadex LH-20 column (80% MeOH) and purified with a silica Lobar A®-column ($CHCl_3$: MeOH = 6 : 1) to give compounds **9** (5 mg), **10** (12 mg) and **11** (25 mg). In turn, fraction B3 (2.8 g) was separated by Sephadex LH-20 column and purified with a silica Lobar A®-column to give compounds **12** (18 mg) and **13** (24 mg). Fraction B4 (0.7 g) was separated over a silica gel column and RP-C_{18} prep. HPLC (50% MeOH) to give compound **14** (15 mg).

Caryophyllene oxide (1) – Colorless oil, $[\alpha]^{20}_D$: –91.0° (*c* 0.25, $CHCl_3$); FAB-MS *m/z*: 243 $[M + Na]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.99 (1H, br d, *J* = 1.5 Hz, H-14a), 4.88 (1H, br d, *J* = 1.5 Hz, H-14b), 2.90 (1H, dd, *J* = 13.0, 4.0 Hz, H-2a), 2.62 (1H, dd, *J* = 10.0, 9.5 Hz, H-9), 2.36 (1H, dq, *J* = 8.0, 4.0 Hz, H-7a), 2.25 (1H, dq, *J* = 8.0, 4.0 Hz, H-6a), 2.12 (2H, tt, *J* = 13.0, 4.0 Hz, H-2a, 7b), 1.78 (1H, t, *J* = 10.0 Hz, H-1), 1.71 (1H, dd, *J* = 10.5, 9.5 Hz, H-10a), 1.64 (1H, t, *J* = 10.5 Hz, H-10b), 1.45 (1H, m, H-2b), 1.33 (2H, m, H-3a, 6b), 1.22 (3H, s, H-15), 1.12 (1H, d, *J* = 14.0 Hz, H-3b), 1.05 (3H, s, H-12), 1.02 (3H, s, H-13); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 152.0 (C-8), 112.9 (C-14), 63.9 (C-5), 60.0 (C-4), 51.0 (C-1), 48.9 (C-9), 40.0 (C-10), 39.4 (C-7), 34.2 (C-11), 30.4 (C-6), 30.1 (C-3), 30.0 (C-12), 27.4 (C-2), 21.8 (C-13), 17.2 (C-15).

Bornyl acetate (2) – Colorless oil, $[\alpha]^{20}_D$: +43.2° (*c* 1.0, $CHCl_3$); FAB-MS *m/z*: 197 $[M + H]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.88-4.91 (1H, m, H-2), 2.34-2.38 (1H, m, H-3b), 2.09 (3H, s, -COOH), 1.92-1.96 (1H, m, H-5b), 1.76 (1H, m, H-6a), 1.67-1.69 (1H, m, H-5a), 1.21-1.34 (2H, m, H-3a), 0.99 (1H, dd, *J* = 13.5, 3.5 Hz, H-4), 0.97 (3H, s, Me-10), 0.96 (3H, s, Me-9), 0.91 (3H, s, Me-8); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 171.6 (C-11), 80.1 (C-2), 48.9 (C-1), 47.9 (C-7), 45.1 (C-4), 36.9 (C-3), 28.2 (C-5), 27.3 (C-6), 21.5 (C-9), 19.9 (C-12), 19.0 (C-8), 13.6 (C-10).

Nerolidol (3) – Colorless oil, $[\alpha]^{20}_D$: +11.2° (*c* 1.75, $CHCl_3$); FAB-MS *m/z*: 223 $[M + H]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 5.92 (1H, dd, *J* = 18.0, 11.0 Hz, H-2), 5.22 (1H, d, *J* = 18.0 Hz, H-1b), 5.21 (1H, d, *J* = 11.0 Hz, H-1a), 1.97-2.08 (2H, m, H-4), 1.97-2.08 (2H, m, H-8), 1.67 (3H, brs, H-15), 1.60 (3H, brs, H-12), 1.59 (3H, brs, H-14), 1.27 (3H, brs, H-13); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 145.2 (C-2), 135.7 (C-7), 131.6 (C-11), 124.4 (C-10), 124.4 (C-6), 111.8 (C-1), 73.6 (C-3), 42.2 (C-4), 39.8 (C-8), 28.0 (C-9), 26.8 (C-5), 25.8 (C-15), 22.9 (C-13), 17.8 (C-14), 16.2 (C-12).

Spathulenol (4) – Colorless oil, $[\alpha]^{20}_D$: +11.2° (*c* 1.05, $CHCl_3$); IR ν_{max} cm^{-1}: 3402, 2904, 1771, 1682, 1636 and 1616 cm^{-1}; FAB-MS *m/z*: 221 $[M + H]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.70 (1H, s, H-10b), 4.68 (1H, s, H-10a), 1.32 (3H, s, H-11), 1.07 (3H, s, H-9), 1.05 (3H, s, H-8), 0.48 (1H, d, *J* = 9.0 Hz, H-1a), 0.46 (1H, d, *J* = 9.0 Hz, H-7b); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 153.8 (C-4), 106.5 (C-10), 81.2 (C-7), 54.6 (C-7a), 53.6 (C-4a), 41.9 (C-6), 39.0 (C-3), 30.1 (C-7b), 28.9 (C-9), 27.7 (C-1a), 26.9 (C-5), 26.3 (C-11), 24.9 (C-2), 20.5 (C-1), 16.6 (C-8).

(–)-Borneol (5) – Colorless oil, $[\alpha]^{20}_D$: +19.2° (*c* 0.4, $CHCl_3$); FAB-MS *m/z*: 155 $[M + H]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.04 (1H, ddd, *J* = 10.0, 3.5, 2.0 Hz, H-2), 2.29 (1H, m, H-4), 1.90 (1H, m, H-3a), 1.75 (1H, m, H-5a), 1.63 (1H, m, H-3b), 1.26 (1H, m, H-6a), 0.97 (1H, dd, *J* = 13.5, 3.5 Hz, H-6b), 0.88 (3H, s, H-10), 0.87 (3H, s, H-9), 0.86 (3H, s, H-8); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 77.6 (C-2), 49.7 (C-1), 48.2 (C-7), 45.3 (C-4), 39.2 (C-3), 28.5 (C-5), 26.1 (C-6), 20.4 (C-9), 18.9 (C-8), 13.5 (C-10).

(+)-5-Endo-hydroxycamphor (6) – Colorless oil, $[\alpha]^{20}_D$: +41.5° (*c* 0.9, $CHCl_3$); ESI-MS *m/z*: 168 $[M]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 4.65 (1H, dddd, *J* = 9.5, 4.5, 4.0, 2.0 Hz, H-5b), 2.73 (1H, d, *J* = 19.5 Hz, H-3a), 2.17-2.22

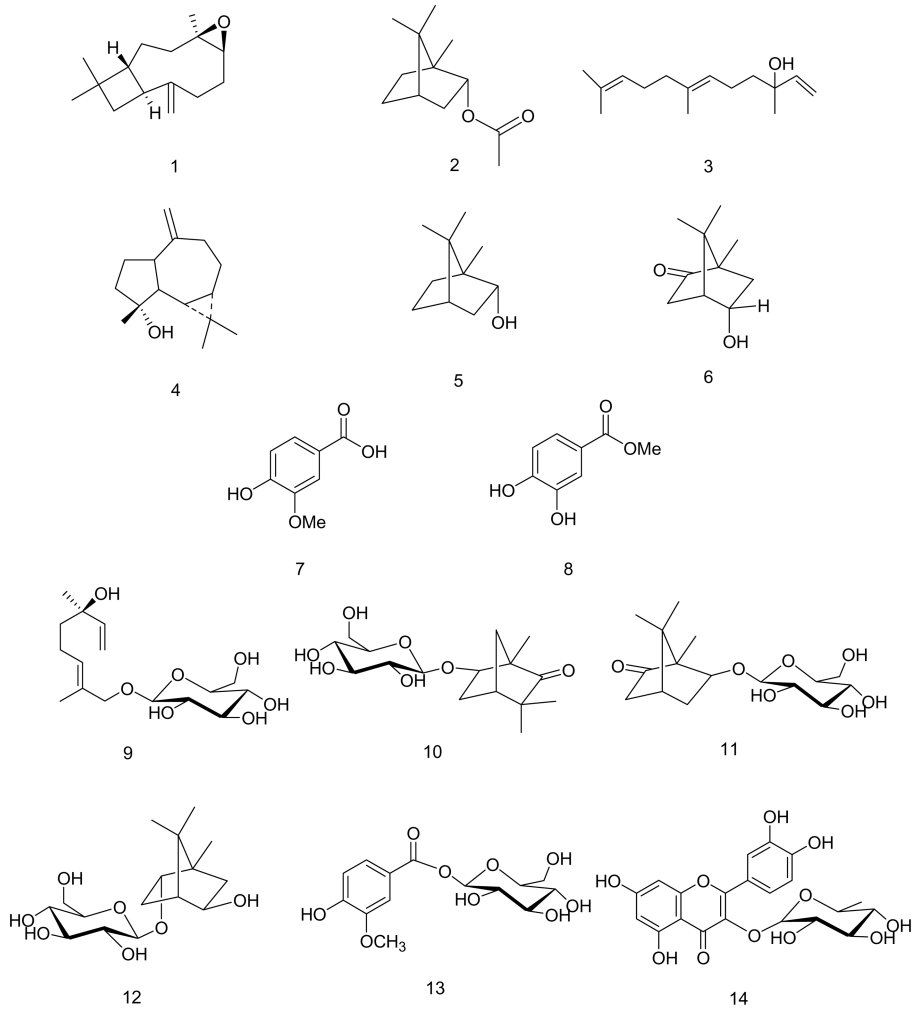

Fig. 1. The structures of **1 - 14** from *A. xanthioides*.

(1H, m, H-4), 2.20 (1H, ddd, J = 19.5, 4.5, 2.0 Hz, H-3b), 2.17 (1H, ddd, J = 14.5, 9.5, 1.5 Hz, H-6b), 1.26 (1H, dd, J = 14.5, 4.0 Hz, H-6a), 1.01 (3H, s, H-8), 0.88 (3H, s, H-10), 0.87 (3H, s, H-9); ^{13}C-NMR (CD_3OD, 125 MHz): δ 218.8 (C-2), 69.7 (C-5), 59.3 (C-1), 49.0 (C-4), 47.8 (C-7), 41.1 (C-6), 34.8 (C-3), 20.5 (C-9), 19.5 (C-8), 9.5 (C-10).

Vanillic acid (7) – Colorless oil, $[\alpha]^{20}_D$: +11.9° (*c* 0.25, MeOH); ESI-MS *m/z*: 168 $[M]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 7.57 (1H, dd, J = 8.0, 1.5 Hz, H-6), 7.56 (1H, d, J = 1.5 Hz, H-2), 6.85 (1H, d, J = 8.0 Hz, H-5), 3.90 (3H, s, -OCH_3); ^{13}C-NMR (CD_3OD, 125 MHz): δ 168.8 (-COOH), 151.4 (C-3), 147.5 (C-4), 124.1 (C-1), 122.0 (C-6), 116.5 (C-2), 114.6 (C-5), 55.6 (-OCH_3).

Protocatechuic acid methyl ester (8) – Colorless oil, $[\alpha]^{20}_D$: +15.9° (*c* 0.2, MeOH); ESI-MS *m/z*: 168 $[M]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 7.43 (1H, d, J = 2.0 Hz, H-6), 7.41 (1H, dd, J = 8.5, 2.0 Hz, H-2), 6.81 (1H, d, J = 8.5 Hz, H-5), 3.84 (3H, s, -OCH_3); ^{13}C-NMR (CD_3OD, 125 MHz): δ 167.6 (-COOH), 150.5 (C-3), 145.0 (C-4), 122.4 (C-1), 121.4 (C-6), 116.2 (C-2), 114.6 (C-5), 51.0 (-OCH_3).

Betulabuside A (9) – Colorless gum, $[\alpha]^{20}_D$: –7.7° (*c* 0.2, MeOH); FAB-MS *m/z*: 333 $[M + H]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 5.95 (1H, dd, J = 17.5, 10.5 Hz, H-7), 5.49 (1H, br t, J = 7.0 Hz, H-3), 5.29 (1H, dd, J = 17.5, 1.5 Hz, H-8a), 5.05 (1H, dd, J = 11.0, 1.5 Hz, H-8b), 4.33 (1H, d, J = 8.0 Hz, H-1') 4.26 (1H, d, J = 11.5 Hz, H-1b), 4.03 (1H, d, J = 11.5 Hz, H-1a), 3.88 (1H, dd, J = 12.0, 2.0 Hz, H-6'b), 3.68 (1H, dd J = 12.0, 5,5 Hz, H-6'a), 3.20-3.38 (4H, m, H-2', H-3', H-4', H-5'), 2.11 (2H, m, H-4), 1.71 (3H, s, H-9), 1.59 (1H, m, H-5), 1.26 (3H, s, H-10); ^{13}C-NMR (CD_3OD, 125 MHz): δ 145.0 (C-7), 131.7 (C-2), 128.9 (C-3), 110.9 (C-8), 101.4 (C-1'), 77.0 (C-3'), 76.7 (C-5'), 74.7 (C-1), 73.9 (C-2'), 72.5 (C-6), 70.5 (C-4'), 61.6 (C-6'), 41.7 (C-5), 26.4 (C-4), 22.2 (C-10), 12.8 (C-9).

(1*R*,4*S*,6*R*)-6-Hydroxyfenchan-2-one-6-*O*-β-D-glucopyranoside (10) – Colorless gum, $[\alpha]^{20}_D$: –17.8° (*c* 0.5, MeOH); FAB-MS *m/z*: 331 $[M+H]^+$; ^{1}H-NMR (Pyridine, 500 MHz): δ 4.73 (1H, d, *J* = 7.5 Hz, H-1'), 4.35 (1H, m, H-6'b), 4.22 (1H, m, H-6'a), 4.17-4.08 (2H, m, H-3', H-4'), 4.07-4.02 (1H, m, H-6), 3.90 (1H, m, H-2'), 3.80 (1H, m, H-5'), 2.25 (1H, m, H-5a), 1.87-1.96 (3H, m, H-4, 5b, 7a), 1.56 (1H, d, *J* = 11.0 Hz, H-7b), 1.26 (3H, s, H-10), 0.85 (3H, s, H-8), 0.75 (3H, s, H-9); ^{13}C-NMR (Pyridine, 125 MHz): δ 221.8 (C-2), 101.9 (C-1'), 78.8 (C-5'), 78.8 (C-3'), 76.6 (C-6), 75.2 (C-2'), 71.7 (C-4'), 62.7 (C-6'), 60.5 (C-1), 47.3 (C-3), 44.7 (C-4), 38.5 (C-7), 36.0 (C-5), 23.9 (C-8), 21.3 (C-9), 11.8 (C-10).

(1*S*,4*R*,6*S*)-6-Hydroxybornan-2-one-6-*O*-β-D-glucopyranoside (11) – Colorless gum, $[\alpha]^{20}_D$: +8.5° (*c* 1.2, MeOH); FAB-MS *m/z*: 331 $[M+H]^+$; ^{1}H-NMR (Pyridine, 500 MHz): δ 4.63 (1H, d, *J* = 8.0 Hz, H-1'), 4.39 (1H, m, H-6'b), 4.26 (1H, m, H-6'a), 4.16-4.02 (2H, m, H-3', H-4'), 3.86 (1H, dd, *J* = 8.0, 4.0 Hz, H-6), 3.85 (1H, m, H-2'), 3.75 (1H, m, H-5'), 2.24 (1H, m, H-5a), 2.13 (1H, m, H-3a), 1.91 (1H, m, H-4), 1.89 (1H, m, H-5b), 1.59 (1H, d, *J* = 11.0 Hz, H-3b), 1.24 (3H, s, H-10), 1.07 (3H, s, H-8), 0.61 (3H, s, H-9); ^{13}C-NMR (Pyridine, 125 MHz): δ 217.6 (C-2), 106.4 (C-1'), 81.1 (C-6), 78.8 (C-3'), 78.5 (C-5'), 75.6 (C-2'), 71.9 (C-4'), 64.3 (C-1), 63.0 (C-6'), 43.3 (C-4), 42.9 (C-3), 40.3 (C-5), 21.7 (C-8), 20.8 (C-9), 6.8 (C-10).

(1*R*,2*S*,4*S*,5*R*)-Angelicoidenol 2-*O*-β-D-glucopyranoside (12) – Colorless gum, $[\alpha]^{20}_D$: –8.7° (*c* 0.85, MeOH); FAB-MS *m/z*: 333 $[M+H]^+$; ^{1}H-NMR (Pyridine, 500 MHz): δ 4.89 (1H, d, *J* = 7.5 Hz, H-1'), 4.39 (1H, br d, *J* = 10.5 Hz, H-2), 4.34 (1H, dd, *J* = 8.0, 3.0 Hz, H-5), 4.32-4.26 (1H, m, H-6'), 4.16-4.02 (2H, m, H-3', H-4'), 3.85 (1H, m, H-2'), 3.75 (1H, m, H-5'), 2.95 (1H, m, H-6a), 2.31 (1H, m, H-3a), 1.97 (1H, m, H-4), 1.73 (1H, m, H-6b), 1.44 (1H, m, H-3b), 1.39 (3H, s, H-8), 1.12 (3H, s, H-10), 0.84 (3H, s, H-9); ^{13}C-NMR (Pyridine, 125 MHz): δ 106.2 (C-1'), 85.1 (C-2), 78.6 (C-3'), 78.3 (C-5'), 75.5 (C-2'), 74.8 (C-6), 71.6 (C-4'), 62.8 (C-6'), 53.4 (C-4), 50.8 (C-1), 47.6 (C-7), 40.1 (C-6), 35.7 (C-3), 21.3 (C-8), 20.1 (C-9), 13.8 (C-10).

1-*O*-Vanilloyl-β-D-glucopyranoside (13) – Colorless gum, $[\alpha]^{20}_D$: –6.0° (*c* 1.15, MeOH); FAB-MS *m/z*: 330 $[M]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 7.62-7.66 (2H, m, H-2, H-6), 6.88 (1H, d, *J* = 8.0 Hz, H-5), 5.70 (1H, d, *J* = 8.0 Hz, H-1'), 3.91 (3H, s, -OCH_3), 3.90 (1H, dd, *J* = 12.0, 2.0 Hz, H-6'a), 3.75 (1H, m, H-6'b), 3.43-3.53 (4H, m, H-2', H-3', H-4', H-5'); ^{13}C-NMR (CD_3OD, 125 MHz): δ 165.5 (C=O), 152.2 (C-4'), 147.6 (C-3'), 124.5 (C-6'), 120.6 (C-1'), 114.8 (C-5'), 112.7 (C-2'), 94.9 (C-1), 77.6 (C-3), 76.9 (C-5), 72.8 (C-2), 69.9 (C-4), 61.1 (C-6), 55.2 (-OCH_3).

Quercetin-3-rhamnopyranoside (14) – Yellow gum, $[\alpha]^{20}_D$: –150.6° (*c* 0.65, MeOH); FAB-MS *m/z*: 465 $[M+H]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 7.35 (1H, d, *J* = 1.5 Hz, H-2'), 7.32 (1H, dd, *J* = 8.0, 2.0 Hz, H-6'), 6.93 (1H, d, *J* = 8.5 Hz, H-5'), 6.37 (1H, d, *J* = 2.0 Hz, H-8), 6.21 (1H, d, *J* = 2.0 Hz, H-6), 5.36 (1H, d, *J* = 1.5 Hz, H-1"), 4.23 (1H, dd, *J* = 3.0, 1.5 Hz, H-2"), 3.77 (1H, dd, *J* = 9.5, 3.0 Hz, H-3"), 3.30-3.42 (2H, m, H-4", H-5") 0.96 (3H, d, *J* = 6.0 Hz, H-6"); ^{13}C-NMR (CD_3OD, 125 MHz): δ 178.4 (C-4), 164.9 (C-7), 162.0 (C-5), 158.1 (C-9), 157.3 (C-2), 148.6 (C-3'), 145.2 (C-4'), 135.0 (C-3), 121.7 (C-1'), 121.6 (C-6'), 115.7 (C-2'), 115.1 (C-5'), 104.6 (C-10), 102.3 (C-1"), 98.7 (C-6), 93.5 (C-8), 72.0 (C-4"), 70.9 (C-3"), 70.8 (C-5"), 70.7 (C-2"), 16.4 (C-6")

Test for cytotoxicity *in vitro* – Sulforhodamin B bioassay (SRB) was used as for cytotoxicity screening (Skehan *et al.*, 1990). The *in vitro* cytotoxicity of each compound against four cultured human tumor cells was assessed at the Korean Research Institute of Chemical Technology. The cell lines used were A549 (non small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), SK-MEL-2 (skin melanoma), and HCT15 (colon cancer cells). Doxorubicin was used as a positive control. The cytotoxicities of doxorubicin against A549, SK-OV-3, SK-MEL-2, and HCT cell lines were ED_{50} 0.007, 0.056, 0.117, and 0.164 μM, respectively.

Results and Discussion

Compounds **1, 3 - 4, 7 - 8, 13 - 14** were identified by comparing the ^{1}H-, ^{13}C-NMR, and MS spectral data with the literature values to be caryophyllene oxide (**1**) (Lee *et al*., 2005), bornyl acetate (**2**) (Zhao *et al*., 2007), nerolidol (**3**) (Miyazawa *et al*., 1995), spathulenol (**4**) (Ulubelen *et al*., 1994), (–)-borneol (**5**) (Orihara *et al*., 1993), (+)-5-endo-hydroxycamphor (**6**) (Miyazawa *et al*., 2004), vanillic acid (**7**) (Sun *et al*., 2006), protocatechuic acid methyl ester (**8**) (Kita *et al*., 1998), 1-*O*-vanilloyl-β-D-glucopyranoside (**13**) (Klick *et al*., 1988), and quercetin-3-rhamnopyranoside (**14**) (Fossen *et al*., 1999). Compounds **6 - 14** were isolated for the first time from this plant. The following describes the structural elucidation of compounds **9 - 12**, which were isolated not so often from natural sources.

Compound **9** was obtained as a colorless gum. From the FAB-MS (*m/z* 333 $[M+H]^+$) and ^{1}H- and ^{13}C-NMR spectral data, the molecular formula of **9** was deduced to be $C_{16}H_{28}O_7$. The ^{1}H-NMR spectrum showed four olefinic protons at δ 5.95 (1H, dd, *J* = 17.5, 10.5 Hz), 5.49 (1H, br t, *J* = 7.0 Hz), 5.29 (1H, dd, *J* = 17.5, 1.5 Hz), and 5.05

(1H, dd, J = 11.0, 1.5 Hz), two oxygenated proton signals at δ 4.26 (1H, d, J = 11.5 Hz) and 4.03 (1H, d, J = 11.5 Hz), and two methyl groups at δ 1.71 (3H, s) and 1.26 (3H, s). The ^{13}C-NMR spectrum demonstrated the presence of 16 carbon signals, consisting of four olefinic carbon signals at δ 145.0, 131.7, 128.9 and 110.9 and two oxygenated carbon signals at δ 74.7 and 72.5. An anomeric carbon signal at δ 101.4 and five oxygenated carbon signals (δ 77.0, 76.7, 73.9, 70.5, and 61.6) suggested the presence of D-glucose (Stephen *et al*., 1977). The coupling constant (J = 8.0 Hz) of the anomeric proton of D-glucose indicated that it was β-form (Stephen *et al*., 1977). These spectral data suggested that **9** was a monoterpene glycoside. The chemical shifts of H-1 (C-1) at δ_H 4.26/4.03 (δ_C 74.7) confirmed that the sugar moiety is located at C-1. Based on further comparison with published data (Calis *et al*., 1995), the structure of **9** was identified as betulabuside A.

Compound **10** was obtained as a colorless gum. From the FAB-MS (*m/z* 331 $[M + H]^+$) and ^{1}H- and ^{13}C-NMR spectral data, the molecular formula of **10** was deduced to be $C_{16}H_{26}O_7$. The ^{1}H-NMR spectrum showed oxygenated methine proton signal at δ 4.02-4.07 (1H, m) and three terminal methyl proton signals at δ 1.26 (3H, s), 0.85 (3H, s), and 0.75 (3H, s). In addition sugar moiety signals at δ 4.35 - 3.80 (6H, m) were observed in the ^{1}H-NMR spectrum. The ^{13}C-NMR spectrum demonstrated the presence of 16 carbon signals, including carbonyl carbon signal at δ 221.8 and three methyl carbon signals at δ 23.9, 21.3 and 11.8. An anomeric carbon signal at δ 101.9 and five oxygenated carbon signals (δ 78.8, 78.8, 75.2, 71.7, 62.7) suggested the presence of D-glucose (Stephen *et al*., 1977). The coupling constant (J = 7.5 Hz) of the anomeric proton of D-glucose indicated to be β-form (Stephen *et al*., 1977) and the HMBC correlation between H-1' (δ_H 4.73) and C-6 (δ_C 76.6) confirmed that the sugar moiety is located at C-6. The stereochemistry of carbon at C-1, 4, and 6 was assigned to be *R*, *S*, and *R* form, respectively on the basis of optical rotation ($[\alpha]^{20}_D$: –17.8°) of **10**, in comparison with published data ($[\alpha]_D$: –48°) (Orihara *et al*., 1994a). Thus, the structure of **10** was identified as (1*R*,4*S*,6*R*)-6-hydroxyfenchan-2-one-6-*O*-β-D-glucopyranoside (Orihara *et al*., 1994a).

Compound **11** was obtained as a colorless gum. From the FAB-MS (*m/z* 331 $[M + H]^+$) and ^{1}H- and ^{13}C-NMR spectral data, the molecular formula of **11** was deduced to be $C_{16}H_{26}O_7$. The ^{1}H-NMR spectrum showed sugar moiety signals at δ 4.63 (1H, d, J = 8.0 Hz), 4.39 (1H, m), 4.26 (1H, m), 4.16-4.02 (2H, m), 3.85 (1H, m), and 3.75 (1H, m), oxygenated methine proton signal at δ 3.86 (1H, dd, J = 8.0, 4.0 Hz) and three terminal methyl proton signals at δ 1.24 (3H, s), 1.07 (3H, s), and δ 0.61 (3H, s). The ^{13}C-NMR spectrum demonstrated the presence of 16 carbon signals, including carbonyl carbon signal at δ 217.6 and three methyl carbon signals at δ 21.7, 20.8, and 6.8. An anomeric carbon signal at δ 106.4, its proton coupling constant (J = 8.0 Hz) and five oxygenated carbon signals (δ 78.8, 78.5, 75.6, 71.9, and 63.0) suggested the presence of β-D-glucose (Stephen *et al*., 1977) and the HMBC correlation between H-1' (δ_H 4.63) and C-6 (δ_C 81.1) confirmed that the sugar moiety is located at C-6. The stereochemistry of oxygenated methine carbon at C-6 was assigned to be *S* form on the basis of the same J values of the H-6 (dd, J = 8.0, 4.0 Hz) and optical rotation ($[\alpha]^{20}_D$: +8.5°) of **11**, in comparison with published data (Orihara *et al*., 1994b). Based on further comparison with published data (Orihara *et al*., 1994b), the structure of **11** was identified as (1*S*,4*R*,6*S*)-6-hydroxybornan-2-one-6-*O*-β-D-glucopyranoside.

Compound **12** was obtained as a colorless gum. From the FAB-MS (*m/z* 333 $[M + H]^+$) and ^{1}H- and ^{13}C-NMR spectral data, the molecular formula of **12** was deduced to be $C_{16}H_{28}O_7$. The ^{1}H- and ^{13}C-NMR spectral data of **12** were similar to those of **11**, except for the absence of carbonyl carbon signal. The ^{1}H-NMR spectrum also showed sugar moiety signals at δ 4.89 (1H, d, J = 7.5 Hz), 4.32 - 4.26 (2H, m), 4.16 - 4.02 (2H, m), 3.85 (1H, m), and 3.75 (1H, m), two oxygenated methine proton signals at δ 4.39 (1H, br d, J = 10.5 Hz), 4.34 (1H, dd, J = 8.0, 3.0 Hz) and three terminal methyl proton signals at δ 1.39 (3H, s), 1.12 (3H, s), and 0.84 (3H, s). The ^{13}C-NMR spectrum demonstrated the presence of 16 carbon signals, including two oxygenated carbon signals at δ 85.1 and 74.8 and three methyl carbon signals at δ 21.3, 20.1 and 13.8. An anomeric carbon signal at δ 106.2, its proton coupling constant (J = 7.5 Hz) and five oxygenated carbon signals (δ 78.6, 78.3, 75.5, 71.6, and 62.8) suggested the presence of β-D-glucose (Stephen *et al*., 1977) and the HMBC correlation between H-1' (δ_H 4.89) and C-2 (δ_C 85.1) confirmed that the sugar moiety is located at C-2. Based on further comparison with its physical and NMR spectral data (Kitajima *et al*., 2003), the structure of **12** was identified as (1*R*,2*S*,4*S*,5*R*)-angelicoidenol 2-*O*-β-D-glucopyranoside.

The isolated compounds were tested *in vitro* for cytotoxicity against four human tumor cells using the SRB assay. Compound **3** exhibited moderate cytotoxicity against SK-OV-3 and SK-MEL-2 (ED_{50}: 26.81 and 9.36 μM, respectively). The compound **4** exhibited weak cytotoxicity against A549, SK-OV-3, SK-MEL-2, and

HCT15 (ED_{50}: 26.93, 27.46, 10.75 and 32.93 μM, respectively).

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