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Cytotoxic Coumarins from the Root of *Angelica dahurica*

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Ten coumarins were isolated from the root of *Angelica dahurica* by repeated silica gel column chromatography. Their chemical structures were elucidated on the basic of physicochemical and spectroscopic data. Among them, oxypeucedanin hydrate acetonide (**7**) was isolated for the first time from this plant. Cytotoxicity of coumarins isolated were determined *in vitro* against L1210, HL-60, K562, and B16F10 tumor cell lines by MTT method. Pangelin (**5**) and oxypeucedanin hydrate acetonide (**7**) showed a potent cytotoxic activity with the IC_{50} values of 8.6 to 14.6 μg/mL against four kinds of tumor cell lines. Other compounds showed the moderate cytotoxic activity or no activity against the tumor cell lines.

Key words: *Angelica dahurica*, Coumarins, Pangelin, Oxypeucedanin hydrate acetonide, Tumor cells, Cytotoxicity

INTRODUCTION

Radix Angelicae Dahuricae, the dried root of *Angelica dahurica* Benth et Hook (Umbelliferae) has long been used in Chinese medicine. It has been frequently prescribed as a sedative and an analgesic (Soka, 1985). As a part of our continuing studies to identify novel antitumor agents from natural sources, the CH_2Cl_2 fraction from MeOH extract of *A. dahurica* was found to have the potent cytotoxic activity in L1210 tumor cell line by MTT method (Mosmann, 1983). Several coumarins, a polyacetylene, and essential oil (Fujiwara *et al.*, 1980; Zhang *et al.*, 1980; Kozawa *et al.*, 1981; Zhou *et al.*, 1987; Kim *et al.*, 1992; Qiao *et al.*, 1996; Lechner *et al.*, 2004; Qiao *et al.*, 1997), and many bioactivities such as anti-staphylococcal activity, anti-inflammatory activity, hepatoprotective activity, anti-oxidative activity, antimicrobial activity, antimutagenic activity (Lechner *et al.*, 2004; Lin *et al.*, 2002; Oh *et al.*, 2002; Tsai *et al.*, 1997; Kwon *et al.*, 1997) have been reported on the root of *A. dahurica*. But no detailed research has been reported about the cytotoxic activity. In this paper, we report the isolation, structure elucidation of ten coumarins, which include one first isolated compound and cytotoxic activity against L1210, HL-60, K562, and B16F10 tumor cell lines.

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MATERIALS AND METHODS

General experimental procedures

Melting points were measured on an electrothermal melting point apparatus, and are uncorrected. The IR spectra were recorded on a Jasco Report-100 IR spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded with BRUKER AVANCE 300 NMR spectrometer in $CDCl_3$ using TMS as the internal standard. MS spectra were measured with JEOL JMSAX505WA mass spectrometer. Column chromatography was carried out using silica gel (Merck, 70-230 mesh). The TLC was performed on a precoated Merck Kieselgel 60 F_{254} plate (0.25 mm) and a Merck RP-18 F_{254} plate (5×10 cm).

Plant material

The root of *Angelica dahurica* were purchased from herb market in Taejeon and the voucher specimen (CNU 1513) were deposited at the herbarium in the College of Pharmacy, Chungnam National University.

Extraction and isolation

The dried root (5 kg) was ground and extracted 3 times with MeOH and obtained the MeOH extract (413 g). The extract was diluted with H_2O to give an aqueous solution. The aqueous solution was subsequently partitioned into CH_2Cl_2 and aqueous fractions. The CH_2Cl_2 extract (187 g) was chromatographed on a silica gel using stepwise gradient elution with the solvents hexane-EtOAc to give 7

fractions (AD-1~AD-7). Silica gel column chromatography of fraction AD-2 (18 g) eluting with hexane-EtOAc (5:1) gave two subfractions (AD-2-1~AD-2-2). Subfraction AD-2-1 was subjected to silica gel column eluting with hexane-EtOAc (6:1) afford compound **1** (174 mg). Subfraction AD-2-2 was subjected to silica gel column eluting with hexane-EtOAc (4:1) to afford compounds **2** (84 mg) and **3** (126 mg). Fraction AD-3 (32 g) was subjected to silica gel column eluting with hexane-EtOAc (5:1) to afford compounds **4** (21 mg), **5** (18 mg), and **6** (34 mg). Silica gel column chromatography of fraction AD-4 (24 g) eluting with hexane-EtOAc (3:1) afforded three subfractions (AD-4-1~AD-4-3). Subfraction AD-4-1 was subjected to silica gel column eluting with hexane-acetone (5:1) to afford compound **7** (9 mg). Subfraction AD-4-2 was subjected to silica gel column eluting with hexane-EtOAc (3:1) to afford compound **8** (63 mg). Silica gel column chromatography of fraction AD-5 (16 g) eluting with hexane-EtOAc (2:1) afforded three subfractions (AD-5-1~AD-5-3). Subfraction AD-5-2 was subjected to silica gel column eluting with hexane-acetone (3:1) to afford compound **9** (56 mg). Subfraction AD-5-3 was subjected to silica gel column eluting with hexane-EtOAc (1:1) to afford compound **10** (18 mg).

Isoimperatorin (1)

White crystal, mp. 105-107°C, IR ν_{max} cm^{-1}: 1729 (C=O), 1628, 1604 (aromatic ring, C=C), 1129 (benzofuran), 821, ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.82 (3H, s, H-6"), 1.72 (3H, s, H-5"), 4.94 (2H, d, *J* = 6.9 Hz, H-2"), 5.56 (1H, t-like, *J* = 6.9 Hz, H-3"), 6.29 (1H, d, *J* = 9.6 Hz, H-3), 6.97 (1H, dd, *J* = 0.9, 2.1 Hz, H-3'), 7.17 (1H, br.s, H-8), 7.61 (1H, d, *J* = 2.1 Hz, H-2'), 8.17 (1H, d, *J* = 9.6 Hz, H-4), ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 18.6 (C-6"), 26.2 (C-5"), 70.2 (C-2"), 94.7 (C-8), 105.4 (C-3'), 108.0 (C-10), 113.0 (C-3), 114.7 (C-6), 119.5 (C-2"), 139.9 (C-4), 140.2 (C-4"), 145.3 (C-2'), 149.4 (C-5), 153.1 (C-9), 158.5 (C-7), 161.7 (C-2).

Osthol (2)

White crystal, mp. 82-83°C, IR ν_{max} cm^{-1}: 1720 (C=O), 1600, 1435 (aromatic ring, C=C), 1110 (benzofuran), ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.68 (3H, s, H-5'), 1.85 (3H, s, H-4'), 3.54 (2H, d, *J* = 7.2 Hz, H-1'), 3.93 (3H, s, 7-OMe), 5.24 (1H, t, *J* = 7.2 Hz, H-2'), 6.23 (1H, d, *J* = 9.6 Hz, H-3), 6.84 (1H, d, *J* = 8.4 Hz, H-6), 7.29 (1H, d, *J* = 8.4 Hz, H-5), 7.61 (1H, d, *J* = 9.6 Hz, H-4), ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 161.7 (C-2), 160.6 (C-7), 153.2 (C-9), 144.1 (C-4), 133.0 (C-3'), 126.6 (C-5), 121.6 (C-2'), 118.4 (C-8), 113.4 (C-10), 113.4 (C-3), 107.8 (C-6), 56.4 (7-OMe), 26.2 (C-5'), 22.3 (C-1'), 18.3 (C-4').

Imperatorin (3)

White crystal, mp. 102, IR ν_{max} cm^{-1}: 1722 (C=O), 1587 (aromatic ring, C=C), 1150 (benzofuran), 838, ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.74 (3H, s, H-6"), 1.76 (3H, s, H-5"), 5.02 (2H, d, *J* = 6.9 Hz, H-2"), 5.60 (1H, t-like, *J* = 6.9 Hz, H-3"), 6.37 (1H, d, *J* = 9.6 Hz, H-3), 6.83 (1H, dd, *J* = 0.9, 2.1 Hz, H-3'), 7.36 (1H, s, H-5), 7.70 (1H, d, *J* = 2.1 Hz, H-2'), 7.76 (1H, d, *J* = 9.6 Hz, H-4). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 18.5 (C-5"), 26.2 (C-6"), 70.5 (C-2"), 107.1 (C-3'), 113.1 (C-5), 115.1 (C-3), 116.9 (C-10), 120.2 (C-3"), 126.2 (C-6), 132.1 (C-8), 140.1 (C-4"), 144.3 (C-9), 144.7 (C-4), 147.0 (C-2'), 149.0 (C-7), 160.9 (C-2).

Psoralen (4)

White crystal, mp. 164-165°C, IR ν_{max} cm^{-1}: 1725 (C=O), 1635, 1577 (aromatic ring, C=C), 1136 (benzofuran), 824, ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 6.39 (1H, d, *J* = 9.6 Hz, H-3), 6.85 (1H, dd, *J* = 0.9, 2.4 Hz, H-3'), 7.49 (1H, br.s, H-8), 7.70 (1H, s, H-5), 7.71 (1H, d, *J* = 2.4 Hz, H-2'), 7.81 (1H, d, *J* = 9.6 Hz, H-4), ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 100.3 (C-8), 106.8 (C-3'), 115.1 (C-3), 115.8 (C-10), 120.2 (C-5), 125.3 (C-6), 144.4 (C-4), 147.3 (C-2'), 152.5 (C-9), 156.8 (C-7), 161.4 (C-2).

Pangelin (5)

White crystal, mp. 135-136, IR ν_{max} cm^{-1}: 3649 (OH), 1717 (C=O), 1624 (aromatic ring, C=C), 1134 (benzofuran), ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.19 (1H, dd, *J* = 9.6 Hz, H-4), 7.61 (1H, d, *J* = 2.4 Hz, H-2'), 7.18 (1H, s, H-8), 6.98 (1H, dd, *J* = 0.9, 2.4 Hz, H-3'), 6.30 (1H, d, *J* = 9.6 Hz, H-3), 5.22 (1H, d, *J* = 0.9 Hz, H-5"), 4.55 (1H, m, H-3"), 4.48 (1H, dd, *J* = 9.6, 3.6 Hz, H-2"), 4.41 (1H, dd, *J* = 9.6, 7.2 Hz, H-2"), 2.33 (1H, s, 3"-OH), 1.86 (3H, s, H-6"), ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 161.4 (C-2), 158.5 (C-7), 153.0 (C-9), 148.9 (C-5), 145.6 (C-2'), 143.7 (C-4"), 139.5 (C-4), 114.6 (C-6), 113.8 (C-5"), 113.4 (C-3) 107.8 (C-10), 105.1 (C-3'), 95.1 (C-8), 76.1 (C-2"), 74.6 (C-3"), 19.1 (C-6").

Bergapten (6)

White crystal, mp. 187-188°C, IR ν_{max} cm^{-1}: 1731 (C=O), 1625, 1580 (aromatic ring, C=C), 1124 (benzofuran), 834, ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 4.28 (3H, s, 5-OCH_3), 6.28 (1H, d, *J* = 9.9 Hz, H-3), 7.03 (1H, d, *J* = 2.4 Hz, H-3'), 7.15 (1H, s, H-8), 7.61 (1H, d, *J* = 2.4 Hz, H-2'), 8.16 (1H, d, *J* = 9.9 Hz, H-4), ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 60.5 (5-OCH_3) 94.3 (C-8), 105.4 (C-3'), 106.8 (C-10), 113.0 (C-3), 113.1 (C-6), 139.6 (C-4), 145.2 (C-2'), 150.0 (C-5), 153.1 (C-9), 158.8 (C-7), 161.6 (C-2).

Oxypeucedanin hydrate acetonide (7)

White crystal, mp. 157-158°C, FAB-MS m/z: 345 $[M+H]^+$, IR ν_{max} cm^{-1}: 1736 (C=O), 1620 (aromatic ring, C=C), 1370, 1340 (C-O), 1120 (benzofuran), 820, ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.19 (1H, d, *J* = 9.6 Hz, H-4), 7.63 (1H, d, *J* = 2.4 Hz, H-2'), 7.20 (1H, s, H-8), 7.00 (1H, dd, *J* = 0.9,

2.4 Hz, H-3'), 6.30(1H, d, J = 9.6 Hz, H-3), 4.55 (1H, dd, J = 7.2, 9.9 Hz, H-2"), 4.43 (1H, dd, J = 4.5, 9.9 Hz, H-2"), 4.24 (1H, dd, J = 4.5, 7.2 Hz, H-3"), 1.51 (3H, s, H-8"), 1.45 (3H, s, H-9"), 1.43 (3H, s, H-10"), 1.23 (3H, s, H-11"), ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 161.3 (C-2), 158.5 (C-7), 153.0 (C-9), 148.8 (C-5), 145.6 (C-2'), 139.4 (C-4), 114.5 (C-6), 113.5 (C-3), 108.7 (C-5"), 107.7 (C-10), 105.1 (C-3'), 95.2 (C-8), 81.7 (C-3"), 80.0 (C-7"), 72.4 (C-2"), 28.9 (C-8"), 27.4 (C-9"), 27.2 (C-10"), 23.4 (C-11").

Oxypeucedanin (8)

White crystal, mp. 142-143°C, IR ν_{max} cm^{-1}: 1720 (C=O), 1602 (aromatic ring, C=C), 1386, 1362 (C-O), ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.35 (3H, s, H-6"), 1.43 (3H, s, H-5"), 3.25 (1H, dd, J = 4.5, 6.6 Hz, H-3"), 4.45 (1H, dd, J = 6.6, 10.8 Hz, H-2a"), 4.61 (1H, dd, J = 4.5, 10.8 Hz, H-2b"), 6.33 (1H, d, J = 9.6 Hz, H-3), 6.96 (1H, dd, J = 0.9, 2.4 Hz, H-3'), 7.22 (1H, *br.s*, H-8), 7.63 (1H, d, J = 2.4 Hz, H-2'), 8.22 (1H, d, J = 9.6 Hz, H-4), ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 19.4 (C-6"), 25.0 (C-5"), 58.7 (C-4"), 61.5 (C-3"), 72.8 (C-2"), 95.4 (C-8), 104.9 (C-3'), 107.9 (C-10), 113.6 (C-3), 114.7 (C-6), 139.3 (C-4), 145.7 (C-2'), 148.8 (C-5), 153.0 (C-9), 158.6 (C-7), 161.4 (C-2).

Xanthotoxin (9)

White crystal, mp. 146-147°C, IR ν_{max} cm^{-1}: 1710 (C=O), 1620, 1586 (aromatic ring, C=C), 1155 (benzofuran), 821, ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 4.29 (3H, s, 8-OMe), 6.38 (1H, d, 9.6 Hz, H-3), 6.83 (1H, d, 2.1 Hz, H-3'), 7.36 (1H, s, H-5), 7.70 (1H, d, 2.1 Hz, H-2'), 7.77 (1H, d, 9.6 Hz, H-4), ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 61.7 (8-OMe), 107.1 (C-3'), 113.3 (C-5), 115.2 (C-3), 116.9 (C-10), 126.5 (C-6), 133.2 (C-8), 143.4 (C-9), 144.7 (C-4), 147.0 (C-2'), 148.1 (C-7), 160.8 (C-2).

Oxypeucedanin hydrate (10)

Pale yellowish crystal, mp. 134-135, IR ν_{max} cm^{-1}: 3400 (OH), 1716 (C=O), 1604 (aromatic ring, C=C), 1389, 1370 (C-O), ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.33 (3H, s, H-6"), 1.38 (3H, s, H-5"), 2.18 (1H, s, 4"-OH), 3.04 (1H, s, 3"-OH), 3.93 (1H, dd, J = 3.0, 7.8 Hz, H-3"), 4.45 (1H, dd, J = 7.8, 9.6 Hz, H-2a"), 4.57 (1H, dd, J = 3.0, 9.6 Hz, H-2b"), 6.26 (1H, d, J = 9.9 Hz, H-3), 7.00 (1H, dd, J = 0.9, 2.4 Hz, H-3'), 7.13 (1H, br.s, H-8), 7.61 (1H, d, J = 2.4 Hz, H-2'), 8.17 (1H, d, J = 9.9 Hz, H-4), ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 25.6 (C-6"), 27.0 (C-5"), 72.1 (C-4"), 74.9 (C-2"), 77.0 (C-3"), 95.1 (C-8), 105.2 (C-3'), 107.6 (C-10), 113.3 (C-3), 114.6 (C-6), 139.5 (C-4), 145.6 (C-2'), 148.9 (C-5), 152.9 (C-9), 158.5 (C-7), 161.6 (C-2).

Tumor cells

The murine leukemia L1210 cell, human leukemia HL-60 cell, human leukemia K562 cell, and murine melanoma B16F10 cell were supplied by Dr. HyeongKyu Lee at Immunomodulator Research Laboratory in KRIBB. Cells were maintained in RPMI 1640 including L-glutamine (JBI) with 10% FBS (JBI) and 2% penicillin-streptomycin (GIBCO). Trypsin-EDTA was used in separating cell from culture flask. All the cell lines were cultured at 37 in an atmosphere of 5% CO_2 incubater. The confluent cells were used for the MTT assay.

Cytotoxicity assay

Cytotoxicity was measured by a modification of the Microculture Tetrazolium (MTT) assay (Mosmann, 1983). Viable cells were seeded in the growth medium (180 μL) into 96 well microtiter plate (1×10^4 cells per each well) and allowed to attach in 37°C, 5% CO_2 incubator. The test sample was dissolved in DMSO and adjusted to the final sample concentrations ranging from 30 μg/mL to 1.875 μg/mL by diluting with the growth medium. Each sample was prepared in triplicate. The final DMSO concentration was adjusted to <0.1%. After allowing 2 h, 20 μL test samples were added to each wells the same concentration DMSO were added in the control group. 48 h after test sample addition, 20 μL MTT (final concentration, 5 mg/mL) was added to the each wells. Two hours later, the plate was centrifuged for 5 minutes in 1500 rpm, the medium was removed and formed formazan crystals were dissolved with 150 μL DMSO. The optical density (O.D.) was measured at 570 nm using a Titertek microplate reader (Multiskan MCC/340, Flow). The IC_{50} value was defined as the concentration of sample needed to reduce a 50% of absorbance relative to the vehicle-treated control.

RESULTS AND DISCUSSION

Repeated silica gel column chromatography of the CH_2Cl_2 fraction of the MeOH extract from *A. dahurica* (root) led to the isolation of ten coumarins (**1-10**). Compounds of **1-6** and **8-10** were identified as isoimperatorin (**1**), osthol (**2**), imperatorin (**3**), psoralen (**4**), pangelin (**5**), bergapten (**6**), oxypeucedanin (**8**), xanthotoxin (**9**), and oxypeucedanin hydrate (**10**) by comparing their spectral and physical data with those previously reported (Saiki *et al.*, 1971; Razdan *et al.*, 1987; Fujioka *et al.*, 1999; Stavri *et al.*, 2003).

Compound **7** was obtained as a white crystal. An $[M+H]^+$ peak at 345 in FAB-MS along with the analysis of ^{13}C-NMR, DEPT spectra showed its molecular formula to be $C_{19}H_{20}O_6$. In the ^{1}H-NMR spectrum of compound **7**, two proton doublet signals (1H, J = 9.6 Hz) at δ 6.30 and 8.19 were assignable to be protons of pyrone ring. A proton doublet signal (1H, J = 2.4 Hz) at δ 7.63 and another proton double of doublet signal (1H, J = 0.9, 2.4

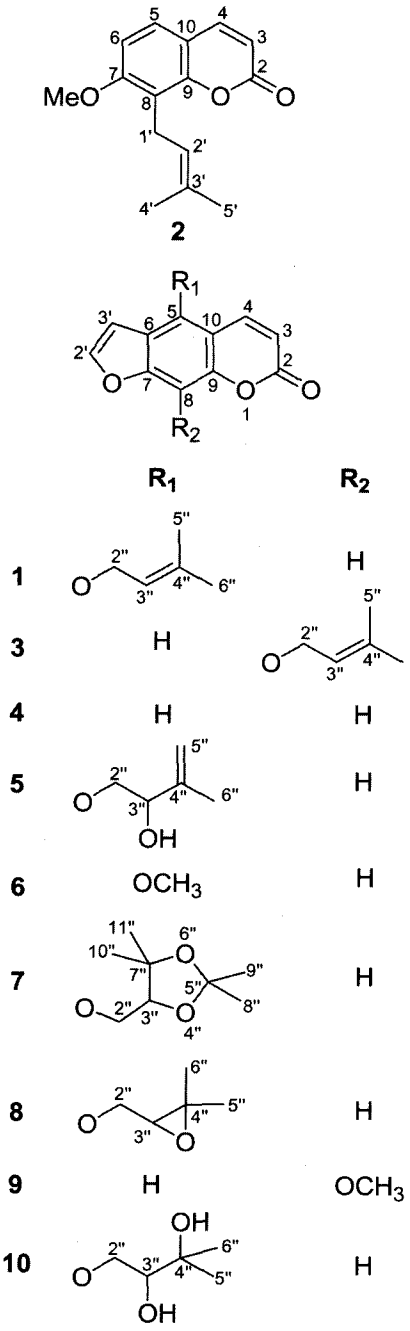

Fig. 1. Structure of compounds **1-10** from the root of *Angelica dahurica*

Hz) at δ 7.00 were attributable to the protons of furan ring. The signal for H-4 was observed rather lower field (δ 8.19) to indicate the absence of proton at C-5 (Harkar et *al.*, 1984). And the signal for H-3′ was observed as a double of doublet (J = 0.9, 2.4 Hz) to mean the occurrence of long-range coupling with H-8 because long-range coupling between H-3' and H-5 in the linear furanocoumarin skeleton was not usually observed (Sasaki et *al.*, 1982). Therefore, a side chain was suggested to be substituted at C-5 position. The presence of (2,2,5,5-tetramethyl-1,3-dioxolan-4-yl)methoxy unit was confirmed by the proton signals observed at δ 4.55 and δ 4.43 (H-2"), δ 4.24 (H-3"), δ 1.51, δ 1.45, δ 1.43, and δ 1.23 (H-8", 9", 10", and 11"). Based on these results and on values previously reported in the literature (Abyshev *et al.*, 1979), compound **7** was identified as oxypeucedanin hydrate acetonide. It has been isolated from this plant for the first time.

Table I. Cytotoxicity of compounds **1-10** from *Angelica dahurica*

Compounds	IC_{50} (μg/mL) [a]			
	L1210	HL-60	K562	B16F10
1	24.5	22.3	20.1	23.5
2	26.4	15.5	25.2	28.2
3	>30	20.2	>30	>30
4	>30	28.9	19.3	>30
5	13.3	14.6	10.1	12.5
6	>30	>30	>30	>30
7	9.4	9.5	8.6	9.8
8	>30	27.5	>30	>30
9	28.9	16.7	24.5	25.6
10	>30	>30	>30	>30
AM [b]	0.8	2.8	1.4	0.9

[a] IC_{50} value was the 50% inhibition concentration and calculated from regression lines using five different concentrations in triplicate experiments.
[b] Adriamycin used in posotive control.

The cytotoxicity assay was carried out against four cancer cell lines, L1210 (murine leukemia cancer), HL-60 (human leukemia cancer), K562 (human leukemia cancer), and B16F10 (murine melanoma cancer) according to the MTT assay described previously (Mosmann, 1983). The results were summarized in Table I. There is no relationship between the cytotoxicity and the structure of isolated coumarins. Among the tested coumarins isolated from the CH_2Cl_2 fraction of *A. dahurica*, compounds **5** and **7** exhibited the most potent cytotoxic activity against all tumor cell lines with IC_{50} values ranging between 8.6 to 14.6 μg/mL while compounds **6** and **10** did not exhibit any cytotoxic activity (IC_{50}, > 30 μg/mL). Up to now there is no report on cytotoxicity of compounds **5** and **7** against tumor cell lines. Compounds **1**, **2**, and **9** exhibited moderate to weak cytotoxic activity against all tumor cell lines with IC_{50} values ranging between 15.5 to 28.9 μg/mL. According to the previous data (Yang *et al.*, 2003), compounds **1**, **2**, and **9** showed moderate to weak cytotoxic effect against HL-60, P-388, CoLo 205 and Hela tumor cell lines with IC_{50} values ranging between 14.9 to 35.3 μg/mL, and in accordance with our data. Interestingly, compound **3** and **8** showed weak cytotoxic activity (IC_{50}, 20.2 and 27.5 μg/mL) against only HL-60 cell line, respectively. Also, com-

pound **4** showed weak cytotoxic activity against only HL-60 and K562 cell lines with IC_{50} values of 28.9 and 19.3 μg/ml, respectively.

These results suggest that the main cytotoxic of the fraction of *A. dahurica* might be attributed to the pangelin (**5**) and oxypeucedanin hydrate acetonide (**7**).

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