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Cytotoxic Phenolic Constituents of *Acer tegmentosum* Maxim

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The chromatographic separation of the MeOH extract from the twigs of *Acer tegmentosum* led to the isolation of ten phenolic compounds. The structures of these compounds were determined using spectroscopic methods as 3,7,3',4'-tetramethyl-quercetin (**1**), 5,3'-dihydroxy-3,7,4'-trimethoxy flavone (**2**), 2,6-dimethoxy-*p*-hydroquinone (**3**), (-)-catechin (**4**), morin-3-*O*-α-L-lyxoside (**5**), *p*-hydroxy phenylethyl-*O*-β-D-glucopyranoside (**6**), 3,5-dimethoxy-4-hydroxy phenyl-1-O-β-D-glucoside (**7**), fraxin (**8**), 3,5-dimethoxy-benzyl alcohol 4-*O*-β-D-glucopyranoside (**9**) and 4-(2,3-dihydroxy propyl)-2,6-dimethoxy phenyl β-D-glucopyranoside (**10**). The compounds were examined for their cytotoxic activity against five cancer cell lines. Compound **3** exhibited good cytotoxic activity against five human cancer cell lines with ED_{50} values ranging from 1.32 to 3.85 μM.

Key words: *Acer tegmentosum*, Acereaceae, Phenolic glycosides, Cytotoxicity

INTRODUCTION

Acer tegmentosum (Acereaceae) has been used in Korean traditional medicine for the treatment of hepatic disorders (Ahn, 1998). Diarylheptanoids (Kubo *et al.*, 1980), rhododendrol glycoside (Kubo *et al.*, 1983), and tannins (Hatano *et al.*, 1990) were isolated from the genus *Acer*. However, the phytochemical constituents and biological activity on *A. tegmentosum* has not been reported. As part of an ongoing study into biological active compounds from Korean natural resources, this work investigated the constituents of the twigs from *A. tegmentosum*, which were collected at Mt. O-Dae, Gangwon Province in October 2002. The twigs of *A. tegmentosum* were extracted with methyl alcohol under reflux. The repeated column chromatographic separation of the extract (140 g) resulted in the isolation of two flavonoids (**1** and **2**), quinone (**3**), tannin (**4**), flavonoid glycoside (**5**), phenolic glycosides (**6**, **7**, **9** and **10**) and coumarin glycoside (**8**). Compounds **4**, **6**, and **8**-**10** were isolated from the genus *Acer* for the first time. The cytotoxic activity of the isolated compounds was tested against five cultured human cancer cell lines. This paper describes the isolation, structure determination and cytotoxic activity of these compounds.

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MATERIALS AND METHODS

General experimental procedures

Melting points were determined on Gallenkamp melting point apparatus and are uncorrected. Optical rotations were measured on a Jasco P-1020 Polarimeter. UV spectra were recorded with a Shimadzu UV 1601 spectrophotometer. NMR spectra were recorded on either a Bruker AMX or a Varian UNITY INOVA 500 NMR spectrometer in $CDCl_3$. EIMS, and FABMS data were obtained on a JEOL JMS700 mass spectrometer. Preparative HPLC used a JAI LC-908 instrument with refractive index detector, UV detector and Alltech Econosil Silica 10 mm column (250 × 22 mm). Open column chromatography was carried out over silica gel (Merck, 70-230) or Sephadex LH-20 (Pharmacia). Low pressure liquid chromatography was carried out over Merck Lichroprep Lobar-A Si 60 (240 ×10 mm) or Lichroprep Lobar-A RP-18 (240×10 mm) column with FMI QSY-0 pump (ISCO).

Plant materials

The twigs from *Acer tegmentosum* were collected at Mt. O-Dae, Korea in October 2002. A voucher specimen of the plants (SKK-2002-002) was deposited at the College of Pharmacy, Sungkyunkwan University, Korea.

Test for cytotoxicity *in vitro*

A sulforhodamin B bioassay (SRB) was used to determine the cytotoxicity of the compounds. (Skehan *et al.*, 1990) The cytotoxic activity of each compound against five cultured human tumor cells was examined at the Korea Research Institute of Chemical Technology; A549 (*non small cell* lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), SK-MEL-2 (skin melanoma), MCF7 (breast; epithelial; pleural effusion adenocarcinoma) and HCT15 (colon cancer cells) *in vitro*.

Extraction and Isolation

The dried and chopped twigs of *A. tegmentosum* (1.5 kg) were extracted three times with methyl alcohol under reflux. The concentrated MeOH extract (140 g) was suspended in distilled water (800 mL) and successively partitioned with *n*-hexane, chloroform and *n*-butanol, followed by evaporation to afford 4 g, 25 g, 45 g of water layer residue, respectively. The water layer residue was re-extracted with 70% acetone (300 mL × 3), which and gave the extract (45 g). The n-hexane fraction was chromatographed over a silica gel column using a gradient solvent system of hexane : EtOAc = 3 : 1~1 : 1 to give four fractions (H1~H4). The H3 fraction (290 mg) was subjected to Sephadex LH-20 column chromatography CH_2Cl_2 : MeOH = 1 : 1 and purified using preparative HPLC (hexane : $CHCl_3$: EtOAc = 5 : 3 : 1, flow rate 2.0 mL/min) to afford compound **1** (20 mg).

The chloroform fraction was chromatographed over silica gel column with CH_2Cl_2 : EtOAc : MeOH = 5 : 3 : 1 as the eluent to give five fractions (C1~C5). The C1 fraction (1.1 g) was subjected to silica gel column chromatography with $CHCl_3$: EtOAc : MeOH = 10 : 1.5 : 0.3 as the eluent to give four subfractions (C11~C14). Subfraction C12 (100 mg) was subjected to a silica gel Lobar®-A column chromatography with hexane : $CHCl_3$: EtOAc = 2 : 2 : 1 as the eluent and purified with preparative HPLC with hexane : CH_2Cl_2 : EtOAc = 2.3 : 2 : 1 as the eluent at a flow rate 2.0 mL/min to afford compounds **2** (20 mg) and **3** (5 mg). The n-butanol fraction was chromatographed over silica gel column with EtOAc : MeOH : H_2O = 10 : 1 : 0.1 as the eluent to give four fractions (B1~B4). Fraction B1 (10 g) was subjected to a silica gel column chromatography with $CHCl_3$: EtOAc : H_2O = 7 : 2 : 0.1 as the eluent to give three subfractions (B11~B13). Subfraction B12 (600 mg) was subjected to Sephadex LH-20 column chromatography (MeOH) to afford compounds **4** (20 mg) and **5** (18 mg). Fraction B13 (1 g) was purified Sephadex LH-20 column chromatography (MeOH) to afford compound **6** (20 mg). Fraction B3 (2.1 g) was subjected to a silica gel column chromatography with $CHCl_3$: MeOH = 5 : 1 as the eluent to give three subfractions (B31~B33). Subfraction B32 (100 mg) was purified with RP C-18 Lobar®-A column chromatography (10% MeOH) to afford compound **7** (8 mg). Fraction B4 (13 g) was subjected to a silica gel column chromatography with EtOAc : MeOH : H_2O = 10 : 3 : 1 as the eluent to give five subfractions (B41 ~ B45). Subfraction B43 (1.7 g) was subjected to a silica gel column chromatography with ($CHCl_3$: MeOH : H_2O = 2 : 1 : 0.1) as the eluent and purified with Sephadex LH-20 column chromatography (80% MeOH) to afford compound **8** (20 mg). The acetone extract (45 g) was chromatographed over a RP C-18 silica gel flash column with 10% MeOH as the eluent to give four fractions (A1~A4). Fraction A2 (3.5 g) was subjected to RP C-18 silica gel column chromatography (10% MeOH) to give four subfractions (A21~A24). Subfraction A22 (90 mg) was subjected to a silica gel Lobar®-A column chromatography with $CHCl_3$: MeOH : H_2O = 4 : 1 : 0.1 as the eluent and Sephadex LH-20 column chromatography (80% MeOH) to afford compounds **9** (20 mg) and **10** (10 mg).

3,7,3',4'-Tetramethyl-quercetin (1)

Yellow powder; mp.: 161°C; FAB-MS m/z : 359 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz) : δ 7.59 (1H, dd, *J* = 2.5, 9.0 Hz, H-6'), 7.42 (1H, d, *J* = 2.5 Hz, H-2'), 7.01 (1H, d, *J* = 9.0 Hz, H-5'), 6.46 (1H, d, *J* = 2.5 Hz, H-8), 6.37 (1H, d, *J* = 2.5 Hz, H-6), 3.98 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 3.85 (3H ,s, OCH_3), 3.84 (3H, s, OCH_3) ; ^{13}C-NMR ($CDCl_3$, 125 MHz) : δ 178.9 (C-4), 165.6 (C-7), 162.3 (C-8a), 156.9 (C-5), 156.0 (C-2), 151.6 (C-4'), 149.0 (C-3'), 139.2 (C-3), 123.2 (C-1'), 122.4 (C-6'), 111.5 (C-2'), 111.1 (C-5'), 106.2 (C-4a), 98.0 (C-6), 92.4 (C-8), 60.4 (OCH_3), 56.3 (OCH_3), 56.2 (OCH_3), 56.0 (OCH_3).

5,3'-Dihydroxy-3,7,4'-trimethoxy flavone (2)

Yellow powder; mp.: 172°C; UV λ_{max} (MeOH) nm (log ε): 256 (2.6), 349 (2.3); FAB-MS m/z : 367 $[M+Na]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz) : δ 7.73 (1H, dd, *J* = 2.5, 8.7 Hz, H-6'), 7.70 (1H, d, *J* = 2.5 Hz, H-2'), 6.98 (1H, d, *J* = 8.7 Hz, H-5'), 6.46 (1H, d, *J* = 2.5 Hz, H-8), 6.37 (1H, d, *J* = 2.5 Hz, H-6), 4.01 (3H, s, OCH_3), 3.89 (6H, s, $OCH_3 \times 2$); ^{13}C-NMR ($CDCl_3$, 125 MHz) : δ 178.0 (C-4), 165.7 (C-7), 162.2 (C-8a), 157.0 (C-5), 155.8 (C-2), 148.9 (C-4'), 145.7 (C-3'), 139.4 (C-3), 123.9 (C-1'), 121.8 (C-6'), 114.6 (C-2'), 110.6 (C-5'), 106.3 (C-4a), 98.1 (C-6), 92.3 (C-8), 60.3 (OCH_3), 56.2 (OCH_3), 56.0 (OCH_3).

2,6-Dimethoxy-*p*-hydroquinone (3)

Yellow powder; mp.: 250°C; UV λ_{max} (MeOH) nm (log ε) : 235 (4.5); EI-MS m/z (rel. int): 169 ($[M+H]^+$, 100); ^{1}H-NMR ($CDCl_3$, 500 MHz) : δ 5.87 (2H, s, H-3,5), 3.84 (6H, s, $OCH_3 \times 2$); ^{13}C-NMR ($CDCl_3$, 125 MHz) : δ 186.73 (C-4), 176.65 (C-1), 157.37 (C-2, 6), 107.45 (C-3, 5), 56.48 ($OCH_3 \times 2$).

(-)-Catechin (4)
Pale yellow powder; $[\alpha]_D$: -14.3° (MeOH; c 0.4); mp. : 234~6°C; UV λ_{max} (MeOH) nm (log ε) : 228 (2.7), 282 (0.7); FAB-MS m/z : 326 $[M]^+$; ^{1}H-NMR (CD_3OD, 500 MHz) : δ 6.84 (1H, d, *J* = 2.0 Hz, H-2'), 6.77 (1H, d, *J* = 7.6 Hz, H-6'), 6.71 (1H, dd, *J* = 2.0, 7.6 Hz, H-5'), 5.93 (1H, d, *J* = 2.3 Hz, H-6), 5.86 (1H, d, *J* = 2.3 Hz, H-8), 4.57 (1H, d, *J* = 7.5 Hz, H-2), 3.98 (1H, dt, *J* = 5.5, 7.5 Hz, H-3), 2.84 (1H, dd, *J* = 5.5, 16.0 Hz, H-4a), 2.50 (1H, dd, *J* = 8.5, 16.0 Hz, H-4b); ^{13}C-NMR (CD_3OD, 125MHz) : δ 157.76 (C-7), 157.52 (C-5), 156.86 (C-8a), 146.19 (C-4'), 146.17 (C-3'), 132.17 (C-1'), 120.02 (C-6'), 116.08 (C-5'), 115.22 (C-2'), 100.80 (C-4a), 96.28 (C-8), 95.49 (C-6), 82.78 (C-2), 68.77 (C-3), 28.44 (C-4).

Morin-3-*O*-β-L-lyxoside (5)
Yellow powder; mp. : 270°C; UV λ_{max} (MeOH) nm (log ε) : 258 (2.7), 351 (2.1); FAB-MS m/z : 457 $[M+Na]^+$; ^{1}H-NMR (CD_3OD, 500 MHz) : δ 7.52 (1H, d, *J* = 2.3 Hz, H-3'), 7.48 (1H, dd, *J* = 2.3, 8.4 Hz, H-5'), 6.89 (1H, d, *J* = 8.4 Hz, H-6'), 6.38 (1H, d, *J* = 2.0 Hz, H-8), 6.20 (1H, d, *J* = 2.0 Hz, H-6), 5.46 (1H, s, H-1"), 4.32 (1H, m, H-2"), 3.90 (1H, m, H-5"), 3.87 (1H, q, *J* = 4.8 Hz, H-4"), 3.51 (2H, t, *J* = 3.6 Hz, H-3", 5"); ^{13}C-NMR (CD_3OD, 125 MHz) : δ 179.98 (C-4), 166.04 (C-7), 163.06 (C-5), 159.34 (C-8a), 158.56 (C-2), 149.84 (C-4'), 146.35 (C-2'), 135.64 (C-3), 123.09 (C-1'), 122.96 (C-6'), 116.83 (C-3'), 116.43 (C-5'), 109.51 (C-4a), 105.61 (C-1"), 99.88 (C-6), 94.77 (C-8), 88.00 (C-2"), 83.30 (C-3"), 78.69 (C-4"), 62.54 (C-5").

***p*-Hydroxy phenylethyl-*O*-β-D-glucopyranoside (6)**
Colorless crytstal; mp. : 220°C; UV λ_{max} (MeOH) nm (log ε) : 225 (3.2), 280 (0.7); FAB-MS m/z : 323 $[M+Na]^+$; ^{1}H-NMR (Pyridine-d_5, 500 MHz) : δ 11.22 (1H, s, OH-4), 7.21 (2H, d, *J* = 8.4 Hz, H-2, 6), 7.12 (2H, d, *J* = 8.4 Hz, H-3, 5), 4.91 (1H, d, *J* = 7.9 Hz, H-1'), 4.56 (1H, d, *J* = 11.8 Hz, H-8a), 4.39 (1H, m, H-8b), 4.33 (1H, q, *J* = 8.2 Hz, H-6'a), 4.26 (2H, m, H-3', 6'b), 4.07 (1H, t, *J* = 7.9 Hz, H-5'), 3.97 (H, m, H-4'), 3.94 (1H, dd, *J* = 7.9, 8.2 Hz, H-2'), 3.02 (2H, t, *J* = 7.6 Hz, H-7); ^{13}C-NMR (Pyridine-d_5, 125 MHz) : δ 157.19 (C-4), 130.38 (C-1), 129.32 (C-2, 6), 116.05 (C-3, 5), 104.57 (C-1'), 78.41 (C-3'), 78.37 (C-5'), 75.02 (C-2'), 71.51 (C-8), 71.01 (C-4'), 62.63 (C-6'), 35.78 (C-7).

3,5-Dimethoxy-4-hydroxy phenyl-1-*O*-β-D-glucoside (7)
Yellow gum; UV λ_{max} (MeOH) nm (log ε) : 283 (2.4); FAB-MS m/z : 341 $[M+Na]^+$; ^{1}H-NMR (DMSO-d_6, 500 MHz) : δ 7.85 (1H, s, OH-4), 6.39 (2H, s, H-2, 6), 5.23 (1H, d, *J* = 4.9 Hz, OH), 5.08 (1H, brs, OH), 5.02 (1H, d, *J* = 4.9 Hz, OH), 4.69 (1H, d, *J* = 7.8 Hz, H-1'), 4.63 (1H, t, *J* = 5.9 Hz, H-6'), 3.72 (6H, s, OCH_3 × 2), 3.44 (1H, dd, *J* = 5.9, 6.4 Hz, H-6'), 3.30 (1H, m, H-5'), 3.24 (1H, d, *J* = 8.3 Hz, H-3'), 3.19 (2H, t, *J* = 8.3 Hz, H-2', 4') ; ^{13}C-NMR (DMSO-d_6, 125 MHz) : δ 150.27 (C-1), 148.12 (C-3, 5), 130.33 (C-4), 101.64 (C-1'), 94.99 (C-2, 6), 77.19 (C-5'), 76.81 (C-3'), 73.29 (C-2'), 70.10 (C-4'), 60.89 (C-6'), 55.81 (OCH_3 × 2).

Fraxin (8)
Yellow powder; mp. : 198°C; UV λ_{max} (MeOH) nm (log ε) : 230 (3.2), 256 (2), 261 (1.5), 348 (2.0); FAB-MS m/z : 393 $[M+Na]^+$; ^{1}H-NMR (Pyridine-d_5, 500 MHz) : δ 7.65 (1H, d, *J* = 9.6 Hz, H-4), 6.87 (1H, s, H-5), 6.26 (1H, d, *J* = 9.6 Hz, H-3), 5.75 (1H, d, *J* = 7.3 Hz, H-1'), 4.48 (1H, dd, *J* = 2.3, 11.8 Hz, H-3'), 4.35 (2H, m, H-5', 2'), 4.32 (3H, m, H-4', 6'), 3.80 (3H, s, OCH_3); ^{13}C-NMR (Pyridine-d_5, 125 MHz) : δ 161.22 (C-2), 146.82 (C-7), 146.56 (C-4), 144.42 (C-6), 144.14 (C-8), 133.53 (C-8a), 112.18 (C-3), 111.7 (C-4a), 106.61 (C-5), 105.29 (C-1'), 78.99 (C-3'), 78.16 (C-5'), 75.46 (C-2'), 71.21 (C-4'), 62.45 (C-6'), 56.21 (OCH_3).

3,5-Dimethoxy-benzyl alcohol 4-*O*-β-D-glucopyranoside (9)
Colorless crystal; mp. : 175~177°C; UV λ_{max} (MeOH) nm (log ε) : 270 (2.1); FAB-MS m/z : 369 $[M+Na]^+$; ^{1}H-NMR (DMSO-d_6, 500 MHz) : δ 6.63 (2H, s, H-2, 6), 5.15 (1H, t, *J* = 5.6 Hz, OH), 4.98 (1H, brs, OH), 4.96 (1H, d, *J* = 3.4 Hz, OH), 4.92 (1H, brs, OH), 4.86 (1H, d, *J* = 7.3 Hz, H-1'), 4.42 (2H, brd, *J* = 4.7 Hz, H-7), 4.26 (1H, t, *J* = 5.6 Hz, OH), 3.74 (6H, s, OCH_3 × 2), 3.58 (1H, m, H-5'), 3.41 (1H, quint, *J* = 5.6 Hz, H-3'), 3.20 (2H, m, H-2', 6'a), 3.15 (1H, t, *J* = 7.3 Hz, H-4'), 3.02 (1H, dq, *J* = 2.1, 5.6 Hz, H-6'b); ^{13}C-NMR (DMSO-d_6, 125 MHz) : δ 153.21 (C-2, 6), 138.93 (C-4), 133.85 (C-1), 105.29 (C-3, 5), 103.45 (C-1'), 77.88 (C-3'), 77.25 (C-5'), 74.88 (C-2'), 70.70 (C-4'), 63.65 (C-7), 61.66 (C-6'), 57.02 (OCH_3 × 2).

4-(2,3-Dihydroxy propyl)-2,6-dimethoxy phenyl β-D-glucopyranoside (10)
Pale yellow gum; UV λ_{max} (MeOH) nm (log ε) : 270 (2.1); FAB-MS m/z : 413 $[M+Na]^+$; ^{1}H-NMR (DMSO-d_6, 500 MHz) : δ 6.52 (2H, s, H-2, 6), 4.83 (1H, d, *J* = 7.7 Hz, H-1'), 3.73 (6H, s, OCH_3 × 2), 3.64 (1H, m, H-8), 3.59 (1H, m, H-5'), 3.41 (1H, quint, *J* = 5.5 Hz, H-3'), 3.29 (1H, t, *J* = 6.2 Hz, H-6'a), 3.20 (1H, m, H-2'), 3.18 (2H, m, H-9), 3.12 (1H, m, H-4'), 3.02 (1H, dd, *J* = 6.2, 8.0 Hz, H-6'b), 2.70 (1H, dd, *J* = 4.4, 13.5 Hz, H-7a), 2.44 (1H, dd, *J* = 8.0, 13.5 Hz, H-7b); ^{13}C-NMR (DMSO-d_6, 125 MHz) : δ 152.91 (C-2, 6), 136.10 (C-4), 133.53 (C-1), 103.63 (C-1'), 108.39 (C-3, 5), 77.86 (C-3'), 77.21 (C-5'), 74.90 (C-2'), 73.07 (C-8), 70.67 (C-4'), 66.17 (C-9), 61.65 (C-6'), 40.75 (C-7).

RESULTS AND DISCUSSION

Compounds **1-5**, **7** and **8** were identified by a comparison of the ^{1}H-, ^{13}C-NMR and MS spectral data reported in the literature as 3,7,3',4'-tetramethyl-quercetin (**1**)

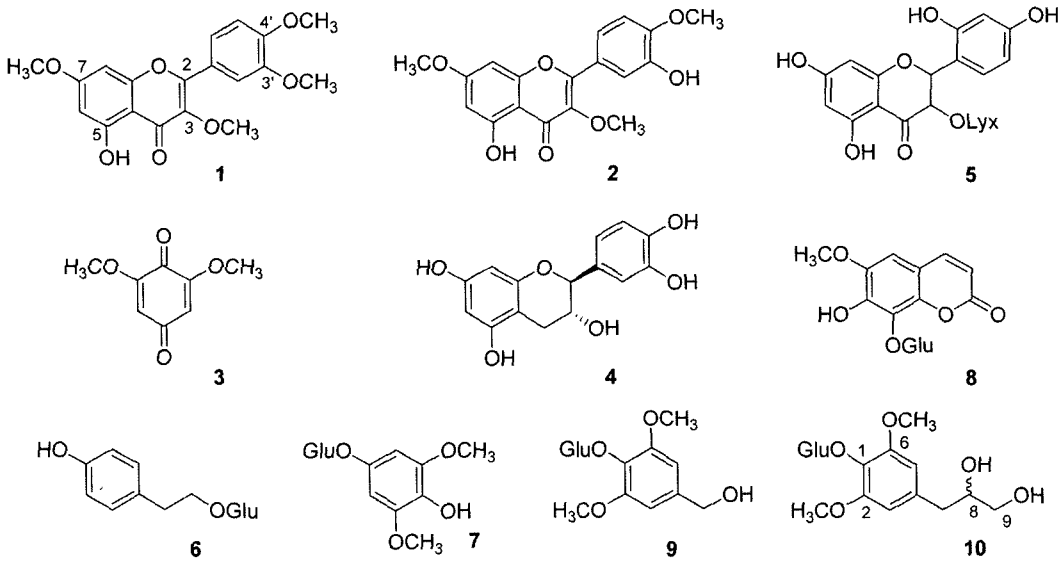

Fig. 1. The structures of compounds **1~10** from *A. tegmentosum*

(Hideyuki *et al.*, 2004), 5,3'-dihydroxy-3,7,4'-trimethoxy-flavone (**2**) (Ying *et al.*, 1989), 2,6-dimethoxy-*p*-hydro-quinone (**3**) (Hideaki et *al.*, 1989), (-)-catechin (**4**) (Adolf *et al.*, 1987; Bilia *et al.*, 1996; Hefeng *et al.*, 1996; Kangi *et al.*, 1987), morin-3-*O*-α-L-lyxoside (**5**) (Hidetoshi *et al.*, 2002), 3,5-dimethoxy-4-hydroxy phenyl-1-*O*-β-D-glucoside (**7**) (Kanji *et al.*, 1990), and fraxin (**8**) (Renmin *et al.*, 2005; Emi *et al.*, 2001). Compounds **4**, **6** and **8-10** were isolated for the first time from the genus *Acer*. The following describes the structure elucidation of compounds **6**, **9** and **10**, which are not common natural constituents and were newly isolated from the genus *Acer*.

Compound **6** was obtained as colorless crystals. FAB-MS, ^{1}H- and ^{13}C-NMR spectroscopy gave a molecular formula of $C_{14}H_{20}O_7$. The ^{1}H-NMR spectrum showed a hydroxyl group signal at δ 11.22 (1H, s), and aromatic signals at δ 7.21 (2H, d, *J* = 8.4 Hz), and δ 7.12 (2H, d, *J* = 8.4 Hz). An ethyl alcohol moiety was observed in the ^{1}H-NMR spectrum at δ 4.56 (1H, d, *J* = 11.8 Hz, H-8a), δ 4.39 (1H, m, H-8b), and δ 3.02 (2H, t, *J* = 7.6 Hz, H-7). The ^{13}C-NMR spectrum demonstrated the presence of 14 carbon signals, which were composed of aromatic carbon signals at δ 157.19, δ 130.38, δ 129.32 (C × 2), and δ 116.05 (C × 2) and an ethyl alcohol moiety at δ 71.51, and δ 35.78. An anomeric carbon signal at δ 104.57 and five oxygenated carbon signals (δ 78.41, δ 78.37, δ 75.02, δ 71.01, and δ 62.63) suggested the presence of D-glucose (Stephen *et al.*, 1977). The coupling constant value (*J* = 7.9 Hz) of the anomeric proton of D-glucose indicated it to be the β-form. The structure of compound **6** was identified as *p*-hydroxy phenylethyl-*O*-β-D-glucopyranoside based on the above consideration and a comparison with the data reported elsewhere (Jorn *et al.*, 2002).

Compound **9** was obtained as colorless crystals. FAB-MS, ^{1}H- and ^{13}C-NMR spectroscopy revealed a molecular formula of $C_{15}H_{22}O_9$. The ^{1}H-NMR spectrum showed an aromatic signal at δ 6.63 (2H, s), and a methyl alcohol moiety at δ 4.42 (2H, brd, *J* = 4.7 Hz). A methoxy signal at δ 3.74 (6H, s), and an anomeric proton signal of sugar at δ 4.86 (1H, d, *J* = 7.3 Hz) were also observed. The ^{13}C-NMR spectral data demonstrated the presence of 15 carbon signals, which were composed of aromatic carbon signals at δ 153.21 (C × 2), δ 138.93, δ 133.85, and δ 105.29 (C × 2), and a methylene signal at δ 63.65. An anomeric carbon signal at δ 103.45 and five oxygenated carbon signals (δ 77.88, δ 77.25, δ 74.88, δ 70.70, and δ 61.66) suggested the presence of D-glucose (Stephen *et al.*, 1977). The structure of compound **9** was identified as 3,5-dimethoxy-benzyl alcohol 4-O-β-D-glucopyranoside based on the above consideration and a comparison with the data reported elsewhere (Junichi *et al.*, 1998).

Compound **10** was obtained as a pale yellow gum. FAB-MS, ^{1}H- and ^{13}C-NMR spectroscopy revealed a molecular formula of $C_{17}H_{26}O_{10}$. The ^{1}H-NMR spectrum showed an aromatic signal at δ 6.52 (2H, s), an anomeric proton signal of sugar at δ 4.83 (1H, d, *J* = 7.7 Hz) and an oxygenated proton signals at δ 3.64 (1H, m), δ 3.59 (1H, m), δ 3.41 (1H, quint, *J* = 5.5 Hz), δ 3.29 (1H, m), δ 3.20 (1H, m), δ 3.18 (2H, m), δ 3.12 (1H, m), and δ 3.02 (1H, dd, *J* = 8.0, 13.5 Hz). In addition, two methylene protons signals at δ 2.70 (H, dd, *J* = 4.4, 13.5 Hz) and δ 2.44 (H, dd, *J* = 8.0, 13.5 Hz) were observed. the ^{13}C-NMR and DEPT spectral data demonstrated the presence of 17 carbon signals, which contained aromatic carbon signals at δ 152.91 (C × 2), δ 136.10 (C), δ 133.53 (C), and δ 108.39 (CH × 2), and a sugar moiety (δ 103.63 (CH), δ

77.86 (CH), δ 77.21 (CH), δ 74.90 (CH), δ 70.67 (CH), and δ 61.65 (CH_2)). The ^1H-^1H COSY spectrum showed correlations between the signal at δ 2.70 (dd, *J* = 4.4, 13.5 Hz, H-7a) and that at δ 2.44 (dd, *J* = 8.0, 13.5 Hz, H-7b) and δ 3.64 (m, H-8), and the signal at δ 3.64 (m, H-8) correlated with the signal at δ 3.18 (2H, m, H-9). This implied the presence of a 1, 2-dihydroxy-propane group. The structure of compound **10** was identified as 4-(2,3-dihydroxy propyl)-2,6-dimethoxy phenyl β-D-glucopyranoside based on the above consideration and a comparison with the data reported elsewhere (Masataka *et al.*, 1992),. However, the configuration of the OH group at C-8 could not be determined due to the instability of the compound and small mount of sample.

Compound **1** showed weak cytotoxicity against SK-OV-3 (ED_{50}: 11.82 μM), and compound **2** exhibited moderate cytotoxicity against A549, SK-OV-3, SK-MEL-2, MCF7 and HCT15 (ED_{50} : 5.92, 3.94, 8.87, 6.10 and 5.21 μM, respectively). However, compound **3** showed good cytotoxicity against A549, SK-OV-3, SK-MEL-2, MCF7 and HCT15 (ED_{50} : 3.71, 1.48, 1.32, 3.85, and 3.70 μM, respectively). The other compounds showed little cytotoxic activity against the cancer cell lines tested (ED_{50} > 30 μM).

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