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Lignans from the Roots of *Berberis amurensis*

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Abstract – Column chromatographic separation of the alcoholic extract from the roots of *Berberis amurensis* yielded eight phenolic constituents including six lignans, hanultarin (**1**), (−)-secoisolariciresinol (**3**), (+)-lyoniresinol (**5**), (+)-syringaresinol (**6**), (+)-syringaresinol-*O*-β-D-glucopyranoside (**7**), liriodendrin (**8**), and two phenylpropanoids, 4-glucosyloxy-3-methoxyphenyl *trans*-propenoic ethyl ester (**2**), *trans*-ferulic acid (**4**). The structures were determined on the basis of NMR spectroscopic data. All isolated compounds (**1 - 8**) were reported for the first time from this source. Compound **1** exhibited moderate cytotoxicity against four human cancer cell lines *in vitro* using sulforhodamin B bioassay.
Key words – *Berberis amurensis*, Lignan, Cytotoxicity

Introduction

Berberis amurensis Rupr. (Berberidaceae) is a decidious shrub widely distributed throughout the middle and northern parts of Korea and other Far East. The roots and woody parts of *B. amurensis* have long been used in folk medicine for the treatment of inflammation and intestinal disorders (Lee *et al*., 1997). Many alkaloids such as berberine, palmatine and berbamine (Tomita *et al*., 1956; Karimov, 1993; Lee *et al*., 1997) were reported from *B. amurensis*, but other constituents have not yet been isolated. In the course of our continuing search for potential bioactive compounds from medicinal plant sources, we have isolated eight phenolic constituents including six lignan derivatives and two phenylpropanoids from the roots of *B. amurensis*. These structures were determined by combined spectroscopic methods including 2D-NMR. All isolated compounds (**1 - 8**) were reported for the first time from this source. Compounds **1 - 8** were tested for their cytotoxic activities against four human cancer cell lines using a SRB assay. Compound **1** exhibited moderate cytotoxicity against four human cancer cell lines *in vitro*.

Experimental

General procedures – Melting point was determined on a Gallenkamp melting point apparatus. Optical rotations were measured using a JASCO P-1020 polarimeter. UV spectrum was obtained on a Varian cary 5000 UV-Visible spectrophotometer. IR spectrum was recorded on a Bruker IFS-66/S FT-IR spectrometer. ESIMS spectra were obtained on an Agilent 1100LC/ MSD trap SL LC/ MS. HRFABMS spectra were obtained on a JEOL JMS700 mass spectrometer. NMR spectra, including COSY, HSQC, HMBC experiments, were recorded on a Varian UNITY INOVA 500 NMR spectrometer. Si gel 60 (Merck, 70 - 230 and 230 - 400 mesh) and RP-C18 silica gel (Merck, 230 - 400 mesh) was used for open column chromatography. Sephadex LH-20 was purchased from Pharmacia Co. Low pressure liquid chromatography was carried out over a Merck Lichroprep Lobar® - A Si 60 (240 × 10 mm) or a Lichroprep Lobar® - A RP-18 (240 × 10 mm) columns with a FMI QSY-0 pump (ISCO). Preparative HPLC used a Gilson 306 pump with Shodex refractive index detector. Silica gel F_{254} plates (Merck) and RP-C18 F_{254s} plates (Merck) were used for TLC. Spots on TLC were detected by UV light and/or by heating after spraying with 10% H_2SO_4 in C_2H_5OH (v/v).

Plant material – The roots of *Berberis amurensis* were collected at Jeju, Korea in December, 2004. A voucher specimen (SKKU-2005-02) was deposited at the College of Pharmacy in Sungkyunkwan University, Korea.

Extraction and isolation – The dried roots of *B. amurensis* (2.5 kg) were extracted with 80% MeOH at room temperature and evaporated *in vacuo* gave 200 g of a residue, which was suspended in water and fractionated

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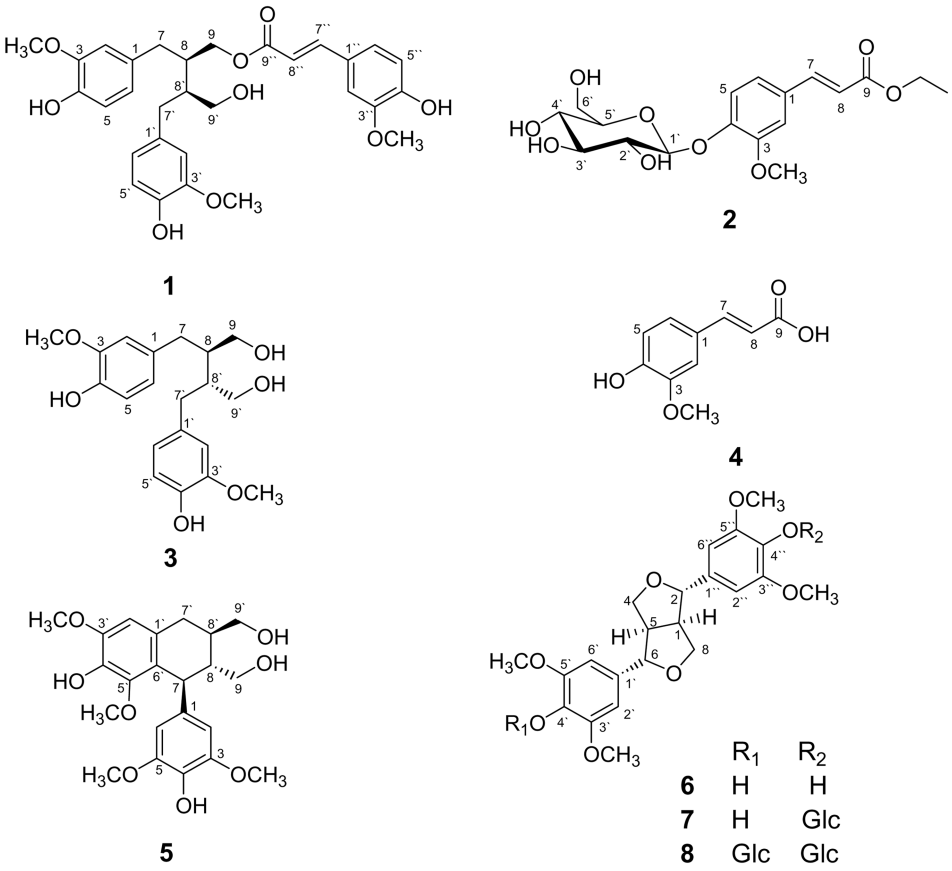

Fig. 1. Structures of compounds **1** - **8** from the roots of *Berberis amurensis*.

with organic solvents to give *n*-hexane (8 g), MCI (6 g), EtOAc (2 g), *n*-BuOH (30 g) and H_2O layer (30 g). The water layer was again extracted with MC to furnish MCII (10 g). The EtOAc fraction (1 g) was separated over a silica gel column with CH_2Cl_2 : MeOH (30 : 1 to 10 : 1) as the eluent to yield eight fractions (E1-E8). Fraction E4 (300 mg) was subjected to Sephadex LH-20 column chromatography (CH_2Cl_2 : MeOH = 1 : 1) and purified with a RP-C_{18} prep. HPLC (40% MeOH - 50% MeOH) to yield compounds **3** (10 mg) and **7** (30 mg). The MCI fraction (3 g) was chromatographed on silica gel, eluting with CH_2Cl_2 gradually increasing the polarity with MeOH (20 : 1 to 1 : 1) to give 10 fractions (M1-M10). Fraction M1 (240 mg) was further separated by LPLC on RP C-18 gel (50% MeOH) and purified with a silica gel prep. HPLC (CH_2Cl_2 : MeOH = 30 : 1) to yield compound **6** (15 mg). Fraction M2 (400 mg) was separated by LPLC on RP C-18 gel (40% MeOH) and purified with a silica gel prep. HPLC with (CH_2Cl_2 : MeOH = 20 : 1) to yield compound **1** (8 mg). Fraction M4 (600 mg) was subjected to Sephadex LH-20 column chromatography (CH_2Cl_2 : MeOH = 1 : 1) and purified with a RP-C_{18} prep. HPLC with 30% MeOH to yield compound **4** (10 mg). Subfraction M8 (500mg) was further separated by LPLC on silica gel (CH_2Cl_2 : MeOH = 10 : 1) and purified with a RP-C_{18} prep. HPLC with 50% MeOH to yield compound **5** (13 mg). The BuOH fraction (10 g) was separated over a silica gel column with CH_2Cl_2 : MeOH (10 : 1 to 1 : 1) as the eluent to yield ten fractions (B1-B10). Fraction B9 (500 mg) was further separated by LPLC on silica gel (CH_2Cl_2 : MeOH = 5 : 1) and purified with a RP-C_{18} prep. HPLC with 50% MeOH to yield compound **8** (35 mg). The MCII fraction (10 g) was also separated over a silica gel column with CH_2Cl_2 : MeOH (15 : 1) as the eluent to yield eight fractions (N1-N8). Fraction N2 was further separated by LPLC on silica gel (CH_2Cl_2 : MeOH = 12 : 1) and purified with a RP-C_{18} prep. HPLC with 50% MeOH to afford compound **2** (15 mg).

Hanultarin (**1**) – Pale yellowish gum, $[\alpha]_D$: –20.3° (*c* 0.1, MeOH); IR (KBr, CH_2Cl_2) ν_{max} cm^{-1}: 3417, 2923, 1695, 1599, 1515, 1459, 1370, 1270, 1160, 1032, 818, 736 cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 326 (4.94), 288 (4.41), 200 (5.18); ESI-MS *m/z*: 561 $[M + Na]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 7.58 (1H, d, *J* = 16.0 Hz, H-7''),

7.19 (1H, d, *J* = 1.5 Hz, H-2"), 7.08 (1H, dd, *J* = 8.0, 1.5 Hz, H-6"), 6.82 (1H, d, *J* = 8.0 Hz, H-5"), 6.69 (2H, dd, *J* = 8.0, 1.5 Hz, H-5, H-5'), 6.65 (1H, d, *J* = 1.5 Hz, H-2'), 6.60 (1H, d, *J* = 2.0 Hz, H-2), 6.57 (2H, dd, *J* = 8.0, 1.5 Hz, H-6, H-6', overlap), 6.36 (1H, d, *J* = 16.0 Hz, H-8"), 4.35 (1H, dd, *J* = 11.0, 6.0 Hz, H-9a), 4.12 (1H, dd, *J* = 11.0, 6.0 Hz, H-9b), 3.91 (3H, s, 3"-OCH_3), 3.75 (6H, s, 3-, 3'-OCH_3), 3.71 (1H, dd, *J* = 11.0, 6.5 Hz, H-9'a), 3.57 (1H, dd, *J* = 11.0, 6.5 Hz, H-9'b), 2.73 (1H, dd, *J* = 13.5, 7.0 Hz, H-7a), 2.65 (2H, d, *J* = 6.5 Hz, H-7', overlap), 2.64 (1H, dd, *J* = 13.5, 7.0 Hz, H-7b), 2.26 (1H, br m, H-8), 2.00 (1H, br m, H-8'); ^{13}C-NMR (CD_3OD, 125 MHz): δ 168.1 (C-9"), 147.7 (C-4"), 148.3 (C-3"), 147.6 (C-3, 3'), 145.7 (C-7"), 144.5 (C-4, 4'), 132.6 (C-1'), 132.1 (C-1), 126.4 (C-1"), 123.0 (C-6"), 121.5 (C-6, 6'), 115.4 (C-5"), 114.7 (C-5, 5'), 114.3 (C-8"), 112.3 (C-2'), 112.2 (C-2), 110.6 (C-2"), 64.8 (C-9), 61.6 (C-9'), 55.3 (C-3"-OCH_3), 55.1 (C-3, 3'-OCH_3), 43.5 (C-8'), 39.5 (C-8), 34.8 (C-7), 34.3 (C-7'); HR-FAB-MS $[M]^+$ *m/z* 538.2212 ($C_{30}H_{34}O_9$, calcd. 538.2203).

4-Glucosyloxy-3-methoxyphenyl *trans*-propenoic ethyl ester (2) – Colorless gum, ESI-MS *m/z*: 407 $[M + Na]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 7.64 (1H, d, *J* = 16.0 Hz, H-7), 7.26 (1H, d, *J* = 1.5 Hz, H-2), 7.20 - 7.18 (2H, m, H-6, H-5, overlap), 6.43 (1H, d, *J* = 16.0 Hz, H-8), 4.98 (1H, d, *J* = 7.5 Hz, H-1'), 4.25 (2H, q, *J* = 7.0 Hz, H-11), 3.90 (3H, s, 3-OCH_3), 3.88 (1H, dd, *J* = 12.0, 2.0 Hz, H-6'a), 3.70 (1H, dd, *J* = 12.0, 5.5 Hz, H-6'b), 3.60-3.40 (4H, m, H-2', H-3', H-4', H-5'), 1.33 (3H, t, *J* = 7.0 Hz, 11-CH_3); ^{13}C-NMR (CD_3OD, 125 MHz): δ 167.7 (C-9), 149.9 (C-3), 148.9 (C-4), 144.7 (C-7), 129.4 (C-1), 122.3 (C-6), 116.4 (C-8), 116.3 (C-5), 111.4 (C-2), 101.1 (C-1'), 77.1 (C-3'), 76.7 (C-5'), 73.7 (C-2'), 70.1 (C-4'), 61.3 (C-6'), 60.4 (C-11), 55.6 (C-3-OCH_3), 13.4 (C-11-CH_3).

(–)-Secoisolariciresinol (3) – Colorless gum, $[\alpha]_D$: –32.2° (*c* 0.1, MeOH); ESI-MS *m/z*: 385 $[M + Na]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 6.67 (2H, d, *J* = 8.0 Hz, H-5, H-5'), 6.61 (2H, d, *J* = 2.0 Hz, H-2, H-2'), 6.56 (2H, dd, *J* = 8.0, 2.0 Hz, H-6, H-6'), 3.75 (6H, s, 3, 3'-OCH_3), 3.60 (4H, m, H-9, H-9'), 2.68 (2H, dd, *J* = 14.0, 7.0 Hz, H-7'a, H-7a), 2.57 (2H, dd, *J* = 14.0, 8.0 Hz, H-7b, H-7'b), 1.92 (2H, br m, H-8, H-8'); ^{13}C-NMR (CD_3OD, 125 MHz): δ 148.9 (C-3, 3'), 145.6 (C-4, 4'), 134.0 (C-1, 1'), 122.9 (C-6, 6'), 115.9 (C-5, 5'), 113.5 (C-2, 2'), 62.3 (C-9, 9'), 56.3 (C-3, 3'-OCH_3), 44.3 (C-8, 8'), 36.2 (C-7, 7').

***trans*-Ferulic acid (4)** – Colorless gum, ESI-MS *m/z*: 193 $[M-H]^-$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 7.61 (1H, d, *J* = 16.0 Hz, H-7), 7.19 (1H, d, *J* = 2.0 Hz, H-2), 7.08 (1H, dd, *J* = 8.5, 2.0 Hz, H-6), 6.82 (1H, d, *J* = 8.0 Hz, H-5), 6.32 (1H, d, *J* = 16.0 Hz, H-8), 3.91 (3H, s, 3-OCH_3); ^{13}C-NMR (CD_3OD, 125 MHz): δ 169.9 (C-9), 149.3 (C-3), 148.2 (C-7), 145.6 (C-4), 126.7 (C-1), 124.1 (C-6), 114.9 (C-8), 114.7 (C-5), 110.6 (C-2), 55.3 (C-3-OCH_3).

(+)-Lyoniresinol (5) – Colorless gum, $[\alpha]_D$: +42.9° (*c* 0.1, MeOH); ESI-MS *m/z*: 443 $[M + Na]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ 6.60 (1H, s, H-2'), 6.41 (2H, s, H-2, H-6), 4.32 (1H, d, *J* = 5.5 Hz, H-7), 3.87 (3H, s, 3'-OCH_3), 3.75 (6H, s, 3, 5-OCH_3), 3.61 (1H, dd, J = 10.0, 5.0 Hz, H-9'a), 3.50 (1H, H-9'b, overlap), 3.50 (2H, d, *J* = 5.0 Hz, H-9, overlap), 3.40 (3H, s, 5'-OCH_3), 2.72 (1H, dd, *J* = 15.0, 5.0 Hz, H-7'a), 2.59 (1H, dd, *J* = 15.0, 11.0 Hz, H-7'b), 1.99 (1H, br m, H-8), 1.64 (1H, br m, H-8'); ^{13}C-NMR (CD_3OD, 125 MHz): δ 147.6 (C-3, 5), 147.2 (C-3'), 146.3 (C-5'), 137.9 (C-1), 137.5 (C-4'), 133.1 (C-4), 128.8 (C-1'), 124.9 (C-6'), 106.3 (C-2'), 105.4 (C-2, 6), 65.3 (C-9'), 62.7 (C-9), 58.7 (C-5'-OCH_3), 55.3 (C-3, 5-OCH_3), 55.2 (C-3'-OCH_3), 47.8 (C-8), 40.9 (C-7), 38.5 (C-8'), 32.2 (C-7').

(+)-Syringaresinol (6) – Colorless gum, $[\alpha]_D$: +4.7° (*c* 0.1, $CHCl_3$); ESI-MS *m/z*: 441 $[M + Na]^+$; ^{1}H-NMR (CD_3OD, 500MHz): δ 6.67 (4H, s, H-2', H-6,' H-2", H-6"), 4.74 (2H, d, *J* = 4.5 Hz, H-2, H-6), 4.28 (2H, dd, *J* = 9.0, 7.0 Hz, H-4a, H-8a), 3.90 (2H, dd, *J* = 9.0, 4.0 Hz, H-4b, H-8b), 3.86 (12H, s, 3', 5', 3", 5"-OCH_3), 3.16 (2H, m, H-1, H-5); ^{13}C-NMR (CD_3OD, 125 MHz): δ 149.5 (C-3', 5', 3", 5"), 136.4 (C-4', 4"), 133.3 (C-1', 1"), 104.7 (C-2', 6', 2", 6"), 87.8 (C-2, 6), 73.0 (C-4, 8), 57.0 (C-3', 5', 3", 5"-OCH_3), 55.7 (C-1, 5).

(+)-Syringaresinol-*O*-*β*-D-glucopyranoside (7) – Colorless gum, $[\alpha]_D$: –20.1° (*c* 0.1, MeOH); ESI-MS *m/z*: 603 $[M + Na]^+$; ^{1}H-NMR (CD_3OD, 500MHz): δ 6.73 (2H, s, H-2", H-6"), 6.67 (2H, s, H-2', H-6'), 4.87 (1H, d, *J* = 7.5 Hz, H-1'"), 4.77 (1H, d, *J* = 4.0 Hz, H-2), 4.72 (1H, d, *J* = 4.5 Hz, H-6), 4.28 (2H, dd, *J* = 15.0, 9.0 Hz, H-4b, H-8b), 3.91 (2H, dd, *J* = 9.0, 3.0 Hz, H-4a, H-8a), 3.86 (6H, s, 3", 5"-OCH_3), 3.85 (6H, s, 3', 5'-OCH_3), 3.79 (1H, dd, *J* = 12.0, 2.5 Hz, H-6'"a), 3.67 (1H, dd, *J* = 12.0, 5.5 Hz, H-6'"b), 3.49 (1H, m, H-2'"), 3.43 (2H, m, H-3'", H-4'"), 3.21 (1H, m, H-5'"), 3.14 (2H, br m, H-1, H-5); ^{13}C-NMR (CD_3OD, 125 MHz): δ 154.6 (C-3", 5"), 149.5 (C-3', 5'), 139.7 (C-1"), 136.3 (C-4'), 135.8 (C-4"), 133.2 (C-1'), 105.5 (C-1'"), 105.0 (C-2", 6"), 104.7 (C-2', 6'), 87.8 (C-6), 87.3 (C-2), 78.5 (C-5'"), 78.0 (C-3'"), 75.9 (C-2'"), 73.1 (C-4), 73.0 (C-8), 71.5 (C-4'"), 62.7 (C-6'"), 57.2 (C-3", 5"-OCH_3), 57.0 (C-3', 5'-OCH_3), 55.9 (C-5), 55.7 (C-1).

Liriodendrin (8) – Pale yellowish powder, mp. 249-250°C; $[\alpha]_D$: –13.5° (*c* 0.1, water); ESI-MS *m/z*: 765 $[M + Na]^+$; ^{1}H-NMR (DMSO-d_6, 500MHz): δ 6.66 (4H, s, H-2', H-6', H-2", H-6"), 4.89 (2H, d, *J* = 10.0 Hz, H-1'", H-1""), 4.67 (2H, d, *J* = 3.0 Hz, H-2, H-6), 4.25 (2H, dd,

J = 11.0, 5.5 Hz, H-4b, H-8b), 3.83 (2H, dd, J = 9.0, 3.0 Hz, H-4a, H-8a), 3.75 (12H, s, 3', 3", 5', 5"-OCH_3), 3.59 (2H, m, H-6'"b, H-6""b), 3.40 (2H, dd, J = 11.5, 6.0 Hz, H-6'"a, H-6""a), 3.20-3.04 (8H, m, H-2'", 3'", 4'", 5'", H-2"", 3"", 4"", 5""), 3.03 (2H, m, H-1, H-5); ^{13}C-NMR (DMSO-d_6, 125 MHz): δ 153.3 (C-3', 3", 5', 5"), 137.8 (C-4', 4"), 134.1 (C-1', 1"), 104.9 (C-2', 2", 6', 6"), 103.4 (C-1'", 1""), 85.8 (C-2, 6), 77.9 (C-5'", 5""), 77.2 (C-3'", 3""), 74.9 (C-2'", 2""), 72.1 (C-4, 8), 70.7 (C-4'", 4""), 61.7 (C-6'", 6""), 57.2 (C-3', 3", 5', 5"-OCH_3), 54.3 (C-1, 5).

Evaluation of cytotoxicity *in vitro* – Cytotoxicity of the isolates were evaluated by sulforhodamin B (SRB) bioassay (Skehan *et al*., 1990). The four human cancer cell lines, A549 (non small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), SK-MEL-2 (skin melanoma), and HCT15 (colon cancer cells), were used for their cytotoxic activities at the Korea Research Institute of Chemical Technology.

Results and Discussion

Column chromatographic separation of the 80% methanol extract from the roots of *B. amurensis*, led to the isolation of eight compounds including six lignan derivatives and two phenylpropanoids. Compounds **2** - **8**, 4-glucosyloxy-3-methoxyphenyl *trans*-propenoic ethyl ester (**2**) (Struijs *et al*., 2008), (−)-secoisolariciresinol (**3**) (Briggs *et al*., 1959; Fonseca *et al*., 1978; Agrawal *et al*., 1982; Achenbach *et al*., 1983; Sugahara *et al*., 2007), *trans*-ferulic acid (**4**) (Xing *et al*., 2003), (+)-lyoniresinol (**5**) (Achenbach *et al*., 1997; Zhang *et al*., 1999), (+)-syringaresinol (**6**) (Deyama *et al*., 1987), (+)-syringaresinol-*O*-β-D-glucopyranoside (**7**) (Kobayashi *et al*., 1985, Shahat *et al*., 2004) and liriodendrin (**8**) (Kobayashi *et al*., 1985, Vermes *et al*., 1991), were identified on the basis of ^{1}H-, ^{13}C-NMR and MS data and by comparison with data in the literatures. Compounds **1**-**8** were isolated for the first time from this plant. The following describes the structural elucidation of compound **1**, which was for the second time isolated from natural sources.

Compound **1** was obtained as a pale yellowish gum. The molecular formula of **1** ($C_{30}H_{34}O_9$) was established by HR-FABMS ([M]$^+$ *m/z* 538.2212, calcd. 538.2203). The structure of **1** was identified through the analyses of ^{1}H-, ^{13}C-NMR and 2D NMR data including ^{1}H-^{1}H COSY and HMBC. The ^{1}H NMR spectrum showed the presence of two olefinic protons at δ 7.58 (1H, d, J = 16.0 Hz) and δ 6.36 (1H, d, J = 16.0 Hz), nine aromatic protons at δ 7.19 (1H, d, J = 1.5 Hz), 7.08 (1H, dd, J = 8.0, 1.5 Hz), 6.82 (1H, d, J = 8.0 Hz), 6.69 (2H, dd, J = 8.0, 1.5 Hz), 6.65 (1H, d, J = 1.5 Hz), 6.60 (1H, d, J = 2.0 Hz), and 6.57 (2H, dd, J = 8.0, 1.5 Hz), three methoxyl proton resonances at δ 3.91 (3H, s,) and 3.75 (6H, s,), eight methylene protons at δ 4.35 (1H, dd, J = 11.0, 6.0 Hz), 4.12 (1H, dd, J = 11.0, 6.0 Hz), 3.71 (1H, dd, J = 11.0, 6.5 Hz), 3.57 (1H, dd, J = 11.0, 6.5 Hz), 2.73 (1H, dd, J = 13.5, 7.0 Hz), 2.65 (2H, d, J = 6.5 Hz), and 2.64 (1H, dd, J = 13.5, 7.0 Hz), two methine protons at δ 2.26 (1H, br m) and 2.00 (1H, br m). The ^{13}C NMR spectrum exhibited 30 carbon resonances, consisting of a ester carbonyl signal at δ 168.1, two olefinic carbon signals at δ 145.7 and 114.3, three methoxyl groups at δ 55.3 and 55.1, two oxygenated carbons at δ 64.8 and 61.6, four methylene carbon signals at δ 43.5, 39.5, 34.8, 34.3, and 18 aromatic carbon signals. The ^{1}H- and ^{13}C-NMR spectra strongly implied that compound **1** could be a lignan derivative. The ^{1}H-^{1}H COSY spectrum showed that the protons at H-7 and at H-9 coupled to the methine proton at δ 2.26 (1H, br m, H-8), and the protons at H-7' and at H-9' coupled to the methine proton at δ 2.00 (1H, br m, H-8'), respectively. Comparison of the partial structure of **1** with those of secoisolariciresinol (**3**) suggested that their structures are closely related. Two olefinic protons at δ 7.58 (1H, d, J = 16.0 Hz, H-7") and 6.36 (1H, d, J = 16.0 Hz, H-8") in the ^{1}H-NMR spectrum, and two olefinic carbon signals at δ 145.7 (C-7") and 114.3 (C-8") and an ester carbonyl carbon at δ 168.1 (C-9") in the ^{13}C-NMR spectrum suggested the presence of (*E*)-feruloyl moiety. In the HMBC spectrum, the correlations between the methylene protons (δ 4.35, H-9a, 4.12, H-9b) and the carbonyl carbon (δ 168.1, C-9") were observed, indicating that the *trans*-feruloyl moiety was attached at C-9. On the basis of the further comparison with literature values (Moon *et al*., 2008), the structure of **1** was determined to be hanultarin.

Compounds **1** - **8** were tested for their cytotoxic activities against four human cancer cell lines using a SRB assay. Compound **1** exhibited moderate cytotoxic activity against the A549, SK-OV-3, SK-MEL-2, and HCT15 cell lines (ED_{50} : 12.2, 12.3, 10.9, and 12.0 μM, respectively), but the other compounds showed little cytotoxicity (ED_{50} > 30 μM).

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References

Achenbach, H., Waibel, R., and Addae-Mensah, I., Lignans and other constituents from *Carissa edulis*. *Phytochemistry* **22**, 749-753 (1983).

Achenbach, H., Benirschke, M., and Torrenegra, R., Alkaloids and other compounds from seeds of *Tabernaemontana cymosa*. *Phytochemistry* **45**, 325-335 (1997).

Agrawal, P.K., and Rastogi, R.P., Two lignans from *Cedrus deodara*. *Phytochemistry* **21**, 1459-1461 (1982).

Briggs, L.H., Cambie, R.C., and Hoare, J.L., Seco-isolariciresinol, a new lignan from *Podocarpus spicatus*. *Tetrahedron Lett*. **4**, 14-15 (1959).

Deyama, T., Ikawa, T., Kitagawa, S., and Nishibe, S., The constituents of *Eucommia ulmoides* Oliv. V. Isolation of dihydroxydehydrodiconiferyl alcohol isomers and phenolic compounds. *Chem. Pharm. Bull*. **35**, 1785-1789 (1987).

Ferreira Fonseca, S., De Paiva Campello, J., Barata, Lauro E.S., and Ruveda, Edmundo A., Carbon-13 NMR spectral analysis of lignans from *Araucaria angustifolia*. *Phytochemistry* **17**, 499-502 (1978).

Karimov, A., Berberis alkaloids. *Chemistry of Natural Compounds* **29**, 415-438 (1993).

Kobayashi, H., Karasawa, H., Miyase, T., and Fukushima, S., Studies on the constituents of Cistanchis Herba. V. Isolation and structures of two new phenylpropanoid glycosides, Cistanosides E and F. *Chem. Pharm. Bull*. **33**, 1452-1457 (1985).

Lee, H.Y., and Kim, C.W., Studies on the constituents of *Berberis amurensis* Ruprecht. *Kor. J. Pharmacogn*. **28**, 257-263 (1997).

Moon, S.S., Rahman, A.A., Kim, J.Y., and Kee, S.H., Hanultarin, A cytotoxic lignan as an inhibitor of actin cytoskeleton polymerization from the seeds of *Trichosanthes kirilowii*. *Bioorg. Med. Chem*. **16**, 7264-7269 (2008).

Shahat, A.A., Abdel-Azim, N.S., Pieters, L., and Vlietinck, A. J., Isolation and NMR spectra of syringaresinol-β-D-glucoside from *Cressa cretica*. *Fitoterapia* **75**, 771-773 (2004).

Skehan, P., Storeng, R., Scudiero, D., Monks, A., Mcmahon, J., Vistica, D., Warren, J.T., Bokesch, H., Kenney, S., and Boyd, M.R., New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst*. **82**, 1107-1112 (1990).

Sugahara, T., Yamauchi, S., Kondo, A., Ohno, F., Tominaga, S., Nakashima, Y., Kishida, T., Akiyama, K., and Maruyama, M., First stereoselective synthesis of meso-secoisolariciresinol and comparison of its biological activity with (+) and (−)-secoisolariciresinol. *Biosci. Biotechnol. Biochem*. **71**, 2962-2968 (2007).

Struijs, K., Vincken, J.P., Verhoef, R., Voragen, A.G.J., and Gruppen, H., Hydroxycinnamic acids are ester-linked directly to glucosyl moieties within the lignan macromolecule from flaxseed hulls. *Phytochemistry* **69**, 1250-1260 (2008).

Stephen, J.P., Louise, N.J., and David, C.P., High-resolution ^{1}H- and ^{13}C-NMR. Spectra of D-glucopyranose, 2-acetamido-2-deoxy-D-glucopyranose, and related compounds in aqueous media. *Carbohydr. Res*. **59**, 19-34 (1977).

Tomita, M., and Kugo, T., Studies on the alkaloids of Berberidaceous plants. VIII. Alkaloids of *Berberis amurensis* Rupr. var. *japonica* (Regel) Rehd. Forma *Bretschneideri* (Rehd.) Ohwi. (2). *Chem. Pharm. Bull*. **4**, 121-123 (1956).

Vermes, B., Seligmann, O., and Wagner, H., Synthesis of biologically active tetrahydrofurofuranlignan-(syringin, pinoresinol)- mono- and bis-glucosides. *Phytochemistry* **30**, 3087-3089 (1991).

Xing, X., Ho, P., Bourquin, G., Yeh, L.A., and Cuny, G.D., Synthesis, stereochemistry confirmation and biological activity evaluation of a constituent from *Isodon excisus*. *Tetrahedron* **59**, 9961-9969 (2003).

Zhang, Z., Guo, D., Li, C., Zheng, J., Koike, K., Jia, Z., and Nikaido, T., Gaultherins A and B, two lignans from *Gaultheria yunnanensis*. *Phytochemistry* **51**, 469-472 (1999).

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