

Isolation of Limonoids and Alkaloids from *Phellodendron amurense* and Their Multidrug Resistance (MDR) Reversal Activity

Yong Deuk Min, Hak Cheol Kwon, Min Cheol Yang, Kyu Ha Lee, Sang Un Choi¹, and Kang Ro Lee

College of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea and ¹Korea Research Institute of Chemical Technology, Daejeon 305-600, Korea

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Three limonoids and five alkaloids were isolated from the chloroform layer of the MeOH extract of the bark of *Phellodendron amurense* (Rutaceae). The structures of the compounds isolated were determined to be obacunone (1), limonin (2), 12 α -hydroxylimonin (3), γ -fagarine (4), oxyberberine (5), canthin-6-one (6), 4-methoxy-N-methyl-2-quinolone (7) and oxypalmatine (8) based on the physicochemical and spectroscopic data. Compounds 3, 5, 7, and 8 were first isolated from the *Phellodendron amurense*. The isolated compounds were then tested for their cytotoxicity against five human tumor cell lines *in vitro* using the SRB method. Compound 5 showed significant cytotoxicity against the five tumor cell lines with ED₅₀ values ranging from 0.30 to 3.0 μ g/mL. The marginal or noncytotoxic compounds (1, 2, 3, 4, and 7) were examined for their P-gp related MDR reversal activities. Compound 1 showed significant P-gp MDR inhibition activity in MES-SA/DX5 and HCT15 cells with an ED₅₀ value of 0.028 μ g/mL and 0.0011 μ g/mL, respectively.

Key words: *Phellodendron amurense*, Rutaceae, Limonoid, Alkaloid, Multidrug resistance

INTRODUCTION

In traditional Chinese Medicine, Phellodendri Cortex (The stem bark of *Phellodendron amurense* Rupr., Rutaceae) has been used to treat dysentery, jaundice, yellow thick foul leukorrhagia, swelling pain in the knees and feet, urinary tract infections, and infections on the body surfaces (Yan *et al.*, 1999). Isoquinoline alkaloids, phenolic compounds, butenolides and limonoids from the bark of this plant have been reported (Kondo *et al.*, 1985; Wada *et al.*, 1990; Miyaki *et al.*, 1992; Kishi *et al.*, 1992; Ida *et al.*, 1994). Indolopyridoquinazoline alkaloids, furoquinoline alkaloids and isoquinoline alkaloids were also extracted from the callus tissues of bark of this plant (Ikuta *et al.*, 1995, 1998a, 1998b).

As part of an ongoing search for multidrug resistance (MDR) reversal compounds from Korean medicinal plants, the present study examined Phellodendri Cortex because the MeOH extract was found to show P-gp mediated MDR reversal activity in human cancer cells.

The chromatographic separation of the chloroform fraction of the MeOH extract from the bark of *P. amurense* led to the isolation of three limonoids and five alkaloids. Their structures were characterized as obacunone (1), limonin (2), 12 α -hydroxylimonin (3), γ -fagarine (4), oxyberberine (5), canthin-6-one (6), 4-methoxy-N-methyl-2-quinolone (7) and oxypalmatine (8) by physicochemical and spectroscopic methods. The compounds were tested for their *in vitro* cytotoxicity against five tumor cell lines using the SRB method. The marginal or non-cytotoxic compounds (1, 2, 3, 4, and 7) were tested for their MDR reversal activity. This paper describes the isolation, structural determination and P-gp expressed MDR reversal activities of the compounds isolated from *P. amurense*.

MATERIALS AND METHODS

General experimental procedures

The melting points were determined on a Gallenkamp melting point apparatus and were uncorrected. The optical rotations were determined using a Jasco P-1020 polarimeter. The Infrared (IR) spectra were recorded on KBr discs using a Bruker Vector 22 FT-IR spectrometer. The ultraviolet (UV) spectra were obtained using a Shimadzu UV-1601 UV/Visible (Japan) and PDA detector

Correspondence to: Kang Ro Lee, Natural Products Laboratory, College of Pharmacy, Sungkyunkwan University, 300 Chonchondong, Jangan-ku, Suwon 440-746, Korea
Tel: 82-31-290-7710, Fax: 82-31-292-8800
E-mail: krlee@skku.ac.kr

(Waters Co.). The nuclear magnetic resonance (NMR) spectra were recorded on Varian VXR-500 and JNM-LA400. The EI-MS data was obtained using a JMS700 spectrometer (Jeol Co.). The LC-ESI-MS/MS data were obtained using a Quattro micro (Waters Co.). The prep-HPLC was performed using a Prep Nova-Pak HR C18 (6 μ m, 19 \times 300 mm) column with a PDA detector (Waters Co., model 2996) and RI detector (Waters Co., model 2414). Silica gel 60 (0.063-0.200 mm, Merck Co.) was used for column chromatography. Kiesel gel 60F₂₅₄ precoated plates (Merck Co.) and RP-18 F_{254s} precoated plates (Merck Co.) were used for thin layer chromatography (TLC). The packing material used for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia Co.).

The following analytical conditions were used for HPLC : detector = PDA; column = RP18, 5 μ m, 4.6 \times 150 mm; eluent = gradient 20% MeOH \rightarrow 90% MeOH (15 min.) \rightarrow 90% MeOH (10 min.).

Plant material

The bark of *P. amurense* was purchased at the Kyungdong herbal market, in March, 2003, Seoul, Korea and a voucher specimen was deposited in the College of Pharmacy at Sungkyunkwan University.

Extraction and isolation

The dried, chopped bark (2.5 kg) was extracted three times with 80% MeOH (6 L \times 3) at room temperature. The resulting extracts (150 g) were suspended with distilled water (3 L), followed by fractionation with *n*-hexane and chloroform. The chloroform layer (40 g) was subjected to silica gel column chromatography using a solvent system of *n*-hexane : ethylacetate : methanol (1 : 1 : 0 \rightarrow 0 : 0 : 1) as the eluant to provide eight sub-fractions (C-1~C-8). Subfraction C-2 (3 g) was recrystallized with a CH₂Cl₂/methanol solvent mixture to give compound **1** (500 mg). Subfraction C-4 (2.5 g) was dissolved in CH₂Cl₂/methanol to give the precipitate (C-4P, 1 g) and supernatant (C-4S, 1.5 g). Precipitate C-4P (1 g) was recrystallized with CH₂Cl₂/methanol to afford compound **2** (280 mg) and a supernatant (C-4P-S, 300 mg). Supernatant C-4P-S (300 mg) was subjected to silica gel column chromatography with the CH₂Cl₂ : methanol solvent system (60:1) as the eluant to afford compound **3** (10 mg). Supernatant C-4S (1.5 g) was chromatographed with a Sephadex LH-20 column (CH₂Cl₂ : methanol = 1 : 1) to give two fractions (C-4S-1 and C-4S-2). Subfraction C-4S-1 was repeatedly purified with a RP-18 Lobar-A column (75% and 80% methanol) and silica Lobar-A column (*n*-hexane: ethylacetate : methanol = 10 : 1 : 0.3) to afford compounds **4** (10 mg) and **5** (10 mg). Subfraction C-4S-2 was purified with RP-18 Lobar-A (85% methanol) and silica Lobar-A

(*n*-hexane : ethylacetate : methanol = 10 : 10 : 0.3) to afford compounds **6** (10 mg), **7** (10 mg) and **8** (5 mg).

Obacunone (1)

Colorless crystal; mp 228~235°C (CH₂Cl₂/MeOH); [α]_D²⁰: -46.4° (*c* 0.24, CHCl₃); IR (neat) ν_{\max} cm⁻¹ : 2988, 1745, 1703, 1630, 1572, 1503, 1451, 1393, 1282; EI-MS *m/z* (rel. int.) : 454 (M⁺, 0.4), 439 (4), 397 (3), 363 (15), 347 (2), 331 (100), 313 (7); ¹H-NMR (500 MHz, CDCl₃) : δ 7.42 (1H, m, H-21), 7.40 (1H, t, *J* = 1.8 Hz, H-23), 6.51 (1H, d, *J* = 11.7 Hz, H-1), 6.36 (1H, dd, *J* = 1.8, 0.9 Hz, H-22), 5.96 (1H, d, *J* = 11.7 Hz, H-2), 5.46 (1H, s, H-17), 3.65 (1H, s, H-15), 2.98 (1H, t, *J* = 14.1 Hz, H-6 β), 2.60 (1H, dd, *J* = 14.1, 5.0 Hz, H-5), 2.28 (1H, dd, *J* = 14.1, 5.0 Hz, H-6 α), 2.14 (1H, br.d, *J* = 8.5, 3.5 Hz, H-9), 1.50 (6H, s, H-29 and H-30), 1.45 (3H, s, H-28), 1.24 (3H, s, H-19), 1.12 (3H, s, H-18); ¹³C-NMR (100 MHz, CDCl₃) : δ 208.10 (C-7), 167.59 (C-3)*, 167.36 (C-16)*, 157.45 (C-1), 143.88 (C-23), 141.70 (C-21), 123.67 (C-2), 120.78 (C-20), 110.44 (C-22), 84.66 (C-4), 78.67 (C-17), 65.74 (C-14), 58.03 (C-5), 54.02 (C-15), 53.65 (C-8), 49.92 (C-9), 43.83 (C-10), 40.60 (C-6), 38.14 (C-13), 33.47 (C-12), 32.73 (CH₃), 27.51 (CH₃), 21.83 (CH₃), 20.16 (CH₃), 17.69 (C-11), 17.14 (CH₃).

* : Assignments may be interchanged.

Limonin (2)

Colorless crystal; mp : 287~293°C (CH₂Cl₂/MeOH); [α]_D²⁰: -127.7° (*c*=0.2, CHCl₃); IR (neat) ν_{\max} cm⁻¹ 2967, 1747, 1714, 1503, 1460, 1285, 1025; EI-MS *m/z* (rel. int.) : 470 (M⁺, 0.6), 454 (4), 412 (13), 347 (100), 329 (15), 135 (22); ¹H-NMR (500 MHz, CDCl₃) : δ 7.41 (1H, m, H-21), 7.40 (1H, m, H-23), 6.34 (1H, m, H-22), 4.76 (1H, d, *J* = 13.2 Hz, H-19 β), 4.46 (1H, d, *J* = 13.2 Hz, H-19 α), 4.04 (2H, br.s, H-1 and H-15), 2.98 (1H, dd, *J* = 16.7, 1.8 Hz, H-2 β), 2.85 (1H, dd, *J* = 15.8, 14.4 Hz, H-6 β), 2.68 (1H, dd, *J* = 16.7, 1.8 Hz, H-2 α), 2.56 (1H, dd, *J* = 12.2, 2.9 Hz, H-9), 2.47 (1H, dd, *J* = 14.4, 3.2 Hz, H-6 α), 2.23 (1H, dd, *J* = 15.8, 3.2 Hz, H-5), 1.29 (3H, s, H-28), 1.18 (3H, s, H-29), 1.17 (3H, s, H-18), 1.07 (3H, s, H-30); ¹³C-NMR (100 MHz, CDCl₃) : δ 206.07 (C-7), 169.05 (C-3), 166.58 (C-16), 143.22 (C-23), 141.11 (C-21), 119.97 (C-20), 109.66 (C-22), 80.30 (C-4), 79.14 (C-1), 77.78 (C-17), 65.67 (C-14), 65.33 (C-19), 60.55 (C-5), 53.85 (C-15), 51.32 (C-8), 48.11 (C-9), 45.94 (C-10), 37.94 (C-13), 36.38 (C-6), 35.64 (C-2), 30.83 (C-12), 30.15 (CH₃), 21.38 (CH₃), 20.69 (CH₃), 18.91 (C-11), 17.61 (CH₃).

12 α -Hydroxylimonin (3)

White powder; [α]_D²⁰: -141.7° (*c* 0.02, CHCl₃); IR (neat) ν_{\max} cm⁻¹ 3526, 2969, 1742, 1505, 1459, 1281, 1022; EI-MS *m/z* (rel. int.) : 486 (M⁺, 3), 471 (3), 440 (19), 429 (14), 363 (100), 345 (11); ¹H-NMR (500 MHz, CDCl₃) : δ 7.52 (1H,

m, H-21), 7.49 (1H, m, H-23), 6.44 (1H, m, H-22), 5.44 (1H, s, H-17), 4.80 (1H, d, $J = 13.2$ Hz, H-19 β), 4.39 (1H, d, $J = 13.2$ Hz, H-19 α), 4.06 (1H, br.s, H-1), 3.93 (1H, br.s, H-12), 3.78 (1H, s, H-15), 1.29 (3H, s, H-29)^a), 1.18 (3H, s, H-18)^a), 1.15 (3H, s, H-28)^a), 0.97 (3H, s, H-30)^a); ¹³C-NMR (100 MHz, CDCl₃): δ 205.79 (C-7), 165.92 (C-16), 169.34 (C-3), 144.45 (C-23), 141.47 (C-21), 119.85 (C-20), 109.33 (C-22), 80.13 (C-4), 78.89 (C-1), 76.54 (C-17), 68.56 (C-12), 65.67 (C-19), 64.22 (C-14), 61.09 (C-5), 52.36 (C-15), 52.11 (C-8), 45.68 (C-10), 44.94 (C-9), 43.49 (C-13), 36.38 (C-6)^b), 35.65 (C-2)^b), 30.30 (C-11), 30.07 (CH₃), 21.39 (CH₃), 16.68 (CH₃), 13.34 (CH₃).

^a), ^b): Assignments may be interchanged.

γ -Fagarine (4)

Pale yellow powder; IR (neat) ν_{\max} cm⁻¹: 3121, 3069, 1622, 1516, 1459, 1395, 1366, 1303, 1262, 1094; UV $\lambda_{\max}^{(\text{MeOH})}$ nm (log ϵ): 311 (3.92), 326 (3.89), 338 (3.84); EI-MS m/z (rel. int.): 229 (M⁺, 100), 228 (57), 200 (54), 199 (24), 156 (17); ¹H-NMR (500 MHz, CDCl₃): δ 7.84 (1H, dd, $J = 8.5$, 1.2 Hz, H-5), 7.64 (1H, d, $J = 2.9$ Hz, furan α H), 7.35 (1H, dd, $J = 8.5$, 7.6 Hz, H-6), 7.06 (1H, dd, $J = 7.6$, 1.2 Hz, H-7), 7.07 (1H, d, $J = 2.9$ Hz, furan β H), 4.44 (3H, s, OCH₃), 4.08 (3H, s, OCH₃); ¹³C-NMR (100 MHz, CDCl₃): δ 163.21 (C-4), 156.85 (C-8), 154.57 (C-2), 143.88 (C- α), 137.52 (C-9), 123.41 (C-6), 119.65 (C-10), 114.06 (C-5), 107.70 (C- β), 104.50 (C-7), 103.87 (C-3), 58.98 (OCH₃), 55.94 (OCH₃).

Oxyberberine (5)

Yellow powder; IR (neat) ν_{\max} cm⁻¹: 2937, 2840, 1647, 1595, 1491, 1472, 1382, 1277, 1228, 1037; UV $\lambda_{\max}^{(\text{MeOH})}$ nm (log ϵ): 218 (4.51), 313 (3.93), 342 (4.10), 367 (3.90), 385 (3.74); EI-MS m/z (rel. int.): 351 (M⁺, 100), 336 (60), 322 (36), 308 (24), 292 (8); ¹H-NMR (500 MHz, CDCl₃): δ 7.32 (1H, d, $J = 8.8$ Hz, H-11), 7.27 (1H, d, $J = 8.8$ Hz, H-12), 7.21 (1H, s, H-13), 6.71 (1H, s, H-1), 6.70 (1H, s, H-4), 6.00 (2H, s, OCH₂O), 4.29 (2H, t, $J = 6.2$ Hz, H-6), 4.01 (3H, s, OCH₃), 3.95 (3H, s, OCH₃), 2.89 (2H, t, $J = 6.2$ Hz, H-5); ¹³C-NMR (100 MHz, CDCl₃): δ 160.10 (C-8), 151.41 (C-9), 149.56 (C-10), 148.43 (C-3), 147.34 (C-2), 135.64 (C-14), 132.36 (C-8a), 130.04 (C-12a), 123.76 (C-14a), 122.26 (C-12), 119.38 (C-4a), 119.04 (C-11), 107.91 (C-4), 104.68 (C-1), 101.40 (OCH₂O), 101.30 (C-13), 61.60 (OCH₃), 56.88 (OCH₃), 39.36 (C-6), 28.70 (C-5).

Canthin-6-one (6)

Pale yellow powder; IR (neat) ν_{\max} cm⁻¹: 3057, 1677, 1634, 1591, 1434, 1390, 1366, 1333, 1241, 1141, 1056; UV $\lambda_{\max}^{(\text{MeOH})}$ nm (log ϵ): 251 (4.53), 260 (4.54), 269 (4.52), 302 (4.32), 346 (4.30), 362 (4.52), 380 (4.49); EI-MS m/z (rel. int.): 220 (M⁺, 100), 192 (52), 165 (5), 139 (3), 97 (2), 86(9), 84(13), 58(3); ¹H-NMR (500 MHz, CDCl₃): δ 8.85

(1H, d, $J = 5.0$ Hz, H-2), 8.70 (1H, d, $J = 7.9$ Hz, H-8), 8.14 (1H, d, $J = 7.9$ Hz, H-11), 8.04 (1H, d, $J = 9.7$ Hz, H-5), 7.99 (1H, d, $J = 5.0$ Hz, H-1), 7.73 (1H, t, $J = 7.9$ Hz, H-9), 7.55 (1H, t, $J = 7.9$ Hz, H-10), 7.00 (1H, d, $J = 9.7$ Hz, H-4); ¹³C-NMR (100 MHz, CDCl₃): δ 159.60 (C-6), 145.91 (C-2), 139.71 (C-4), 139.40 (C-7a), 136.34 (C-3a), 132.14 (C-11c), 130.93 (C-9), 130.37 (C-11b), 128.98 (C-5), 125.69 (C-10), 124.49 (C-11a), 122.72 (C-11), 117.38 (C-8), 116.42 (C-1).

4-Methoxy-N-methyl-2-quinolone (7)

Colorless powder; mp: 62~94; IR (neat) ν_{\max} cm⁻¹: 3449, 1639, 1586, 1503, 1461, 1392, 1327, 1236, 1119; UV $\lambda_{\max}^{(\text{MeOH})}$ nm (log ϵ): 229 (4.73), 269 (3.84), 279 (3.83), 318 (3.77), 333 (3.61); EI-MS m/z (rel. int.): 189 (M⁺, 100), 174 (43), 158 (6), 146 (23), 132 (16), 91 (6), 77 (13); ¹H-NMR (400 MHz, CDCl₃): δ 7.99 (1H, dd, $J = 5.3$, 1.0 Hz, H-8), 7.60 (1H, ddd, $J = 5.7$, 4.8, 1.0 Hz, H-6), 7.35 (1H, dd, $J = 5.7$, 0.6 Hz, H-5), 7.24 (1H, ddd, $J = 5.3$, 4.8, 0.6 Hz, H-7), 6.06 (1H, s, H-3), 3.97 (3H, s, OCH₃), 3.70 (3H, s, NCH₃); ¹³C-NMR (100 MHz, CDCl₃): δ 163.82 (C-2), 162.64 (C-4), 139.75 (C-9), 131.18 (C-5), 123.34 (C-7), 121.61 (C-6), 116.50 (C-10), 114.01 (C-8), 96.49 (C-3), 55.79 (OCH₃), 29.03 (NCH₃).

Oxypalmatine (8)

Yellow powder; EI-MS m/z (rel. int.): 367 (M⁺, 100), 352 (60), 338 (32), 324 (17), 308 (12), 294 (3), 280 (4); ¹H-NMR (500 MHz, CDCl₃): δ 7.33 (1H, d, $J = 8.8$ Hz, H-11), 7.30 (1H, d, $J = 8.8$ Hz, H-12), 7.23 (1H, s, H-13), 6.76 (1H, s, H-1), 6.73 (1H, s, H-4), 4.32 (2H, t, $J = 6.2$ Hz, H-6), 4.02 (3H, s, OCH₃), 3.99 (3H, s, OCH₃), 3.96 (3H, s, OCH₃), 3.94 (3H, s, OCH₃), 2.93 (2H, t, $J = 6.2$ Hz, H-5); ¹³C-NMR (100 MHz, CDCl₃): δ 160.25 (C-8), 151.39 (C-9), 150.14 (C-3), 149.67 (C-10), 148.46 (C-2), 135.67 (C-14), 132.46 (C-8a), 128.55(C-12a), 122.39 (C-14a), 122.12 (C-12), 119.40 (C-4a), 119.13 (C-11), 110.53 (C-4), 107.65 (C-1), 100.86 (C-13), 61.61 (OCH₃), 56.93 (OCH₃), 56.28 (OCH₃), 56.05 (OCH₃), 39.41 (C-6), 28.28 (C-5).

Cytotoxicity test *in vitro*

The sulforhodamin B assay (SRB) was used for the cytotoxicity test. The activity of the compounds was tested at several concentrations against five cultured human tumor cell lines *in vitro* (Skehan *et al.*, 1990); A549 (non small cell lung adenocarcinoma), SK-OV-3 (ovarian), SK-MEL-2 (skin melanoma), XF498 (CNS) and HCT15 (colon).

MDR reversal activity

The human ovarian cancer cell line, SK-OV-3; the human colorectal cancer cell line, HCT15 cells; the human uterine sarcoma cell line, MES-SA; the human MDR uterine sarcoma cell line, MES-SA/DX5; were provided by the

National Cancer Institute (NCI), and were maintained in the Korea Research Institute of Chemical Technology (KRICT). The SK-OV-3 cells did not express P-gp and were not multidrug resistant cancer cells. Meanwhile, HCT15 cells and MES-SA/DX5 cells showed high level P-gp expression. The cell cultures were conducted with RPMI1640 medium supplied with 5% FBS previously reported (Choi *et al.*, 1996). The cells were incubated with serial dilutions of paclitaxel in the presence or absence of each isolated compound (10 μ M) or verapamil (10 μ M) for 72 h. The procedure for calculating the survival fractions was identical to that of the cytotoxicity assay. In this assay, the controls contained each isolated compound or verapamil without paclitaxel.

RESULTS AND DISCUSSION

Obacunone (1) (Sugimoto *et al.*, 1988a), limonin (2) (Sugimoto *et al.*, 1988a, 1988b), γ -fagarine (4) (Robertson *et al.*, 1963; Narasimhan *et al.*, 1974) and canthin-6-one (6) (Ohmoto *et al.*, 1976; Koike *et al.*, 1985) were identified by a comparison of their spectral data (UV, IR, MS, 1 H-NMR, 13 C-NMR) with those reported in the literature.

Compound 3 was obtained as colorless powder. The IR spectrum showed the presence of OH group at 3526 cm^{-1} and C=O group at 1742 cm^{-1} , respectively. The molecular ion peak of compound 3 in the EI-MS spectrum was m/z 486. The 1 H- and 13 C-NMR spectra of compound 3 were

almost same as those of compound 2. However, the major differences were the H-12 proton chemical shift at δ 3.93, and the C-12 carbon chemical shift at δ 68.56 in compound 3, indicating the presence of an OH group at C-12. Based on the above data and the literature survey, the structure of compound 3 was determined to be 12 α -hydroxylimonin. The NMR and physical data of compound 3 was in good agreement with those in reported literature (Sugimoto *et al.*, 1988a).

Compound 5 was obtained as a yellow powder and tested positive on the dragendorff reagent. The IR spectrum showed amide carbonyl group at 1647 cm^{-1} . The molecular ion peak of compound 5 in the EI-MS spectrum was observed at m/z 351 as the base peak. The 1 H-, 13 C-NMR and IR spectral data suggested compound 5 to be an 8-oxo-protoberberine derivative (Patra *et al.*, 1987). The 1 H-NMR spectrum showed H-11 and H-12 signals of the protoberberine D-ring at δ 7.32 and 7.27 (d, J = 8.8 Hz), H-13 signal of the C-ring at δ 7.21 (s), H-1 and H-4 signals of the A-ring at δ 6.70 (s) and 6.71 (s) and H-5 and H-6 signals of the isoquinoline B-ring at δ 2.89 and 4.29 (2H, t, J = 6.2 Hz), respectively. The 1 H-NMR spectrum also showed two methoxyl groups (δ 3.95 and 4.01), an amide carbonyl (C-8, δ 160.10) and a dioxymethylene group (-OCH₂O-, δ 101.40). Based on the above spectral data, the structure of compound 5 was determined to be oxyberberine. The NMR data and physical data of compound 5 were in good agreement with those reported in the

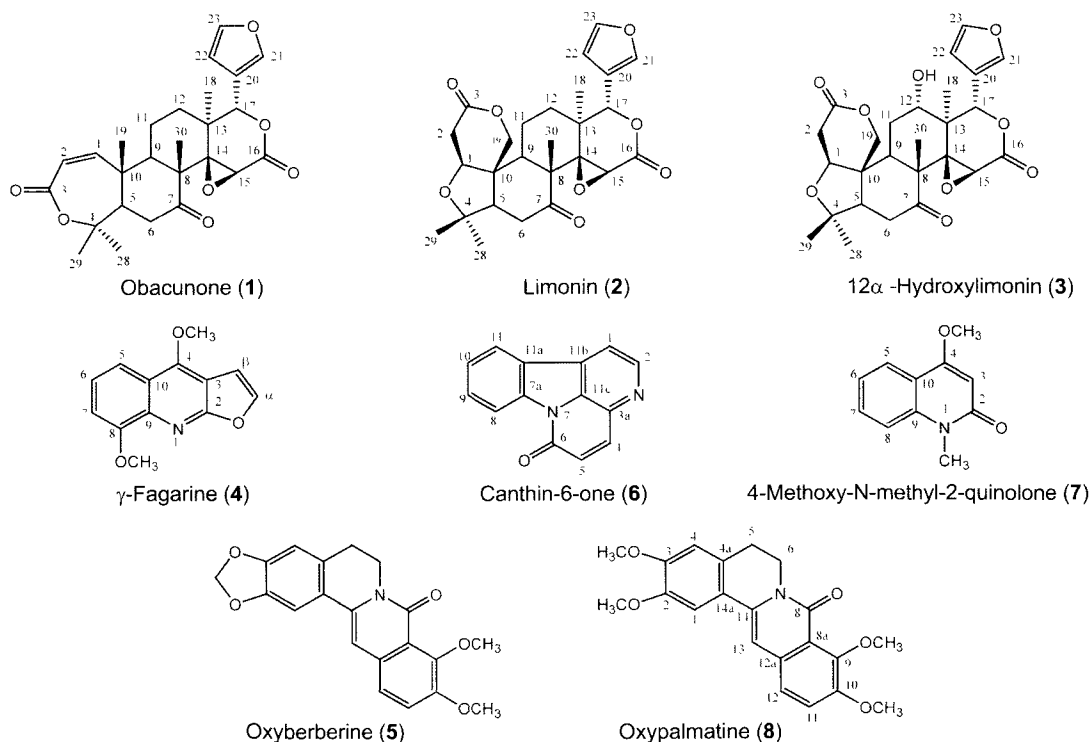


Fig. 1. The Structures of compounds 1-8 from the bark of *P. amurense*

literature (Pinho *et al.*, 1992).

Compound **7** was obtained as a colorless powder and tested positive to the dragendorff reagent. The molecular ion peak of compound **7** in EI-MS spectrum showed *m/z* 189 as the base peak. The ¹H-NMR spectrum showed a H-3 olefinic proton at δ 6.06 (1H, s, H-3), and aromatic ring system protons at δ 7.24 (1H, ddd, *J* = 5.3, 4.8, 0.6 Hz, H-7), 7.35 (1H, dd, *J* = 5.7, 0.6 Hz, H-5), 7.60 (1H, ddd, *J* = 5.7, 4.8, 1.0 Hz, H-6), and 7.99 (1H, dd, *J* = 5.3, 1.0 Hz, H-8). The ¹³C-NMR spectrum showed quinolone ring carbons at δ 163.82 (C-2), 162.64 (C-4), 139.75 (C-9), 131.18 (C-5), 123.34 (C-7), 121.61 (C-6), 116.50 (C-10), 114.01 (C-8), and 96.49 (C-3). From the above spectral data and literature survey, the structure of compound **7** was determined to be 4-methoxy-N-methyl-2-quinolone. The NMR and physical data of compound **7** were in good agreement with the reported literature (Nayar *et al.*, 1971).

Compound **8** was obtained as a yellow powder and tested positive to the dragendorff reagent. The molecular ion peak of compound **8** in EI-MS spectrum was observed as the base peak at *m/z* 367. The ¹H-, ¹³C-NMR spectra of compound **8** were similar to those of compound **5**. The differences were the presence of four methoxyl groups at δ 3.94, 3.96, 3.99 and 4.02, and the absence of a dioxy-methylene group in the ¹H-NMR spectrum of compound **8**. The result of the spectral data suggested compound **8** to be an 8-oxo-protoberberine derivative (Patra *et al.*, 1987). The structure of compound **8** was determined to be oxypalmatine. The NMR data and physical data of compound **8** were in good agreement with the reported literature (Pinho *et al.*, 1992).

Compounds **3**, **5**, **7**, and **8** have not yet reported from this plant.

Compounds **1**, **2**, **3**, **4**, and **7** showed little cytotoxicity against the human cancer cell lines, A549, SK-OV-3, SK-MEL-2, XF498 and HCT15. However, compound **5**

Table I. Cytotoxic activities of compounds (**1-8**) isolated from *Phellodendron amurense*

Compounds	ED ₅₀ (μg/mL)				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	25.46	>30.0	19.71	>30.0	>30.0
2	>30.0	>30.0	>30.0	>30.0	>30.0
3	>30.0	>30.0	>30.0	>30.0	>30.0
4	22.41	27.06	>30.0	>30.0	>30.0
5	1.82	1.17	0.34	3.15	2.93
6	-	-	-	-	-
7	23.19	32.27	>30.0	27.14	>30.0
8	-	-	-	-	-
Doxorubicin	0.16	0.38	0.04	0.04	0.82

* - : not tested

Table II. MDR reversal activities of compounds **1-4** and **7** isolated from *Phellodendron amurense*

Compounds	ED ₅₀ (μg/mL)		
	SK-OV-3	HCT15	MES-SA/DX5
Paclitaxel (P)	0.0004	0.1130	0.817
P + 1	0.0003	0.0011	0.028
P + 2	0.0003	0.0210	0.392
P + 3	0.0007	0.0180	0.449
P + 4	0.0004	0.0100	0.264
P + 7	0.0003	0.0170	0.335
P + verapamil	0.0007	0.0003	0.022

showed significant cytotoxicity against the five tumor cell lines with an ED₅₀ value ranging from 0.3 to 3 μg/mL (Table I). The noncytotoxic compounds were tested for their MDR reversal activity in the P-gp nonexpressed (non-MDR) cell line, SK-OV-3 cell, and the P-gp expressed MDR cell line, MES-SA/DX5 cell and HCT15 cell,. Compounds **1**, **2**, **3**, **4** and **7** showed P-gp MDR inhibition and very weak cytotoxicity to the non-MDR cell line (SK-OV-3). In particular, the P-gp MDR inhibition activity of compound **1** was similar to verapamil, with an ED₅₀ value of 0.028 μg/mL and 0.0011 μg/mL in the MES-SA/DX5 and HCT15 cells, respectively (Table II).

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