

Phytochemical Constituents from the Stem Bark of *Phellodendron amurense* Rupr.

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Three limonoids and two phenolics were isolated from stem bark of *Phellodendron amurense* Rupr. (Rutaceae) and were defined clearly as calodendrolide (1), obacunone (2), limonin (3), methyl 4-hydroxybenzoate (4), and syringin (5) based on NMR spectroscopy results obtained with the aid of X-ray crystallographic analysis. This is the first report on the isolation of calodendrolide (1) from this plant.

Key words: *Phellodendron amurense* Rupr., Rutaceae, limonoid, calodendrolide, X-ray analysis

Phellodendron amurense Rupr., belonging to the family Rutaceae, is well known as an oriental folk medicine. In Korea, China, and Japan, the bark of this plant, in particular, is used as a crude drug for controlling intestinal function, and as an anti-pyretic and anti-inflammatory drug. Recently reported biological activities of the *P. amurense* Rupr. extracts are anti-inflammatory,^{1,2)} anti-gastric ulcer,^{3,4)} and repression for experimental hypertension in rats,⁵⁾ and the major chemical constituents of the crude drug reported so far are limonoids^{6,7)} and alkaloids.^{8,9)} Limonoids have attracted attention due to their insect antifeedant, growth-regulating activity,¹⁰⁾ and, more recently, anticarcinogenic and antitumorogenic activities. In particular, obacunone (2) and limonin (3) showed limonoid insect antifeedant activity.¹⁰⁾ Moreover, syringin (5) in phenolic compounds is effective for the treatment of psychogenic behavior disorder, hypnosis-inducing action, antihypersensitivity effect, and anti-inflammatory effect. Methyl 4-hydroxybenzoate (4) is employed as a preservative of food products, cosmetics, and pharmaceuticals.¹⁹⁾ Herein, we report the isolation and structural elucidation of three limonoids, calodendrolide (1), obacunone (2), and limonin (3), among which compound 1 has isolated for the first time from this species. We also report the isolation of two phenolics, methyl 4-hydroxybenzoate (4) and syringin (5). The structure of compound 2 was defined clearly with the aid of X-ray crystallographic analysis.

Materials and Methods

Materials. The stem bark of *P. amurense* Rupr. was collected in July 2003, in Gyeongsangnam-do Southern Forest Research Center (SFRC), Jinju, Korea and identified at

Gyeongsangnam-do Southern Forest Research Center.

Instruments. Melting points were measured on a Thomas Scientific Capillary Melting point Apparatus and are uncorrected. IR and UV spectra were recorded on a Bruker IFS66 infrared Fourier transform spectrophotometer (KBr) and a Beckman DU650 spectrophotometer, respectively. ¹H- and ¹³C-NMR at 500 and 125 MHz, respectively, and 2D-NMR data were obtained on a Bruker AM 500 spectrometer in CDCl₃ and CD₃OD. EIMS was obtained on a JEOLJMS-700 mass spectrometer.

Extraction and isolation. The air-dried stem bark of *P. amurense* Rupr. (2.0 kg) were cut into pieces and extracted at room temperature with MeOH (5 L × 3) for 7 days. The methanolic extract was then evaporated *in vacuo* to give a crude extract (500 g). The concentrated extract was suspended in water:MeOH (9 : 1) mixture and extracted successively with hexane (1 L), CHCl₃ (1 L), and BuOH (1.5 L). The CHCl₃ extract (40 g) was chromatographed over silica gel using hexane:EtOAc and CHCl₃ : MeOH gradient to give 16 fractions (P1-P16). The fourth fraction (P4) (800 mg) was subjected to the silica gel column chromatography with hexane:ether (30 : 1 → 2 : 1) to afford 8 subfractions. Subfraction 4-5 was subjected to silica gel chromatography (hexane : ether = 25 : 1 → 5 : 1) and was subsequently purified by recrystallization (hexane-ether) to yield calodendrolide (1) (18 mg). The fraction P10 (1.2 g) was subjected to silica gel column chromatography with hexane:acetone (10 : 1 → 2 : 1), and purified by second flash silica gel column using a gradient of solvent polarity hexane:acetone to give obacunone (2) (85 mg) and methyl 4-hydroxybenzoate (4) (13 mg). Fraction P11 (2.5 g) was chromatographed over silica gel at stationary phase using CHCl₃ : acetone gradient (30 : 1 → 2 : 1) to afford 12 subfractions. Limonin 3 (180 mg) was purified from subfractions 3-7 by recrystallization using a mixture of CHCl₃ : acetone. The BuOH extract (35 g) was chromatographed on silica gel, eluting with CHCl₃ : MeOH,

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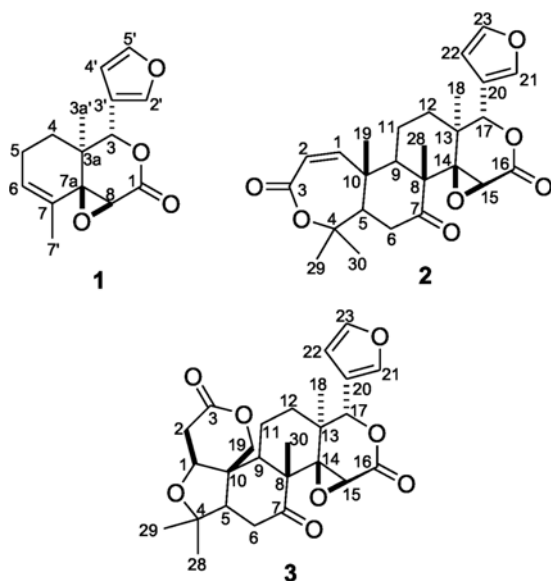


Fig. 1. Structures of compounds 1-3.

and six fractions were collected on the basis of TLC profiles. Fraction 5-6 was subjected to silica gel column chromatography CHCl_3 : MeOH (40 : 1 \rightarrow 1 : 1) to give eight subfractions. The subfraction 2 was purified with silica gel chromatography CHCl_3 : MeOH (20 : 1 \rightarrow 8 : 1) as syringin (**5**) (20 mg).

Calodendrolide (1): amorphous powder; mp 148-150°C; UV (CHCl_3) λ_{max} (log e) 241 (2.2) nm; IR (KBr) ν_{max} 3441, 3187, 2918, 2860, 1742 cm^{-1} ; ^1H NMR (CDCl_3 , 500 MHz) δ 0.98 (3H, s, H-3a), 1.44 (1H, br. dd, J = 12.8, 5.8 Hz, H-4a), 1.61 (3H, s, 7), 1.64 (1H, br. dd, J = 12.8, 6.4 Hz, H-4b), 2.19-2.13 (1H, m, H-5a), 2.26-2.21 (1H, m, H-5b), 3.97 (1H, s, H-8), 5.52 (1H, s, H-3), 5.99 (1H, m, H-6), 6.38 (1H, m, H-2), 7.41 (1H, br. t, J = 1.8 Hz, H-4), 7.42 (1H, s, H-5); ^{13}C NMR (CDCl_3 , 125 MHz) δ 13.7 (C-3a), 17.3 (C-7), 22.4 (C-5), 28.1 (C-4), 37.7 (C-3a), 55.4 (C-8), 65.8 (C-7a), 77.8 (C-3), 110.4 (C-2), 120.5 (C-3), 126.6 (C-7), 133.3 (C-6), 141.5 (C-4), 143.4 (C-5), 168.1 (C-1).

Obacunone (2): colorless prisms; mp 227-230°C; UV (CHCl_3) λ_{max} (log e) 241 (2.64) nm; IR (KBr) ν_{max} 3417, 3121, 3018, 2992, 2949, 1727, 1702, 1650 cm^{-1} ; ^1H NMR (CDCl_3 , 500 MHz) δ 1.12 (3H, s, H-18), 1.25 (3H, s, H-28), 1.45 (3H, s, H-29), 1.50 (3H, s, H-30), 1.51 (3H, s, H-19), 1.89 (4H, m, H-11, H-12), 2.15 (1H, m, H-9), 2.29 (1H, dd, J = 14.0, 5.0 Hz, H-6a), 2.61 (1H, dd, J = 14.1, 5.0 Hz, H-5), 2.99 (1H, dd, J = 14.1 Hz, 5.0 Hz, H-6b), 3.65 (1H, s, H-15), 5.46 (1H, s, H-17), 5.95 (1H, d, J = 11.8 Hz, H-2), 6.37 (1H, t, J = 1.0 Hz, H-21), 6.53 (1H, d, J = 11.8 Hz, H-1), 7.40 (1H, br. t, J = 1.7 Hz, H-22), 7.42 (1H, br. H-23); ^{13}C NMR (CDCl_3 , 125 MHz) δ 16.7 (C-19), 17.2 (C-28), 19.6 (C-11), 21.3 (C-18), 27.0 (C-29), 32.2 (C-30), 32.9 (C-12), 37.7 (C-13), 40.1 (C-6), 43.4 (C-10), 49.4 (C-9), 53.2 (C-8), 53.5 (C-15), 57.5 (C-5), 65.4 (C-14), 78.2 (C-17), 84.2 (C-4), 110.0 (C-21), 120.4 (C-20), 123.1 (C-2), 141.2 (C-22), 143.4 (C-23), 157.1 (C-1), 166.9 (C-16), 167.1 (C-3), 207.7 (C-7).

Limonin (3): amorphous powder; mp 297-300°C; UV

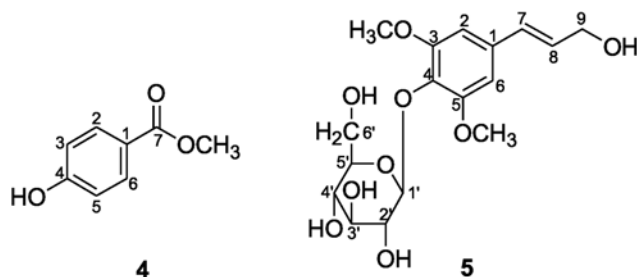


Fig. 2. Structures of compounds 4-5.

(CHCl_3) λ_{max} (log e) 231 (2.93), 236 (2.99), 238 (2.14), 279 (1.56) nm; IR (KBr) ν_{max} 3448, 2969, 2940, 2889, 1757, 1706, 1626 cm^{-1} ; ^1H NMR (CDCl_3 , 500 MHz) δ 1.08 (3H, s, H-30), 1.18 (3H, s, H-18), 1.18 (3H, s, H-28), 1.29 (3H, s, H-29), 1.48-1.54 (1H, m, H-12a), 1.75-1.83 (2H, m, H-11a, H-12b), 1.86-1.93 (1H, m, H-11b), 2.23 (1H, dd, J = 15.8, 3.2 Hz, H-5), 2.46 (1H, dd, J = 14.5, 3.3 Hz, H-6a), 2.56 (1H, dd, J = 12.5, 2.8 Hz, H-9), 2.68 (1H, dd, J = 16.8, 1.9 Hz, H-2a), 2.86 (1H, dd, J = 15.8, 14.7 Hz, H-6b), 2.97 (1H, dd, J = 16.8, 3.8 Hz, H-2b), 4.04 (1H, t, J = 1.4 Hz, H-1), 4.05 (1H, s, H-15), 4.47 (1H, d, J = 13.1 Hz, H-19a), 4.77 (1H, d, J = 13.1 Hz, H-19b), 5.47 (1H, s, H-17), 6.34 (1H, m, H-20), 7.40 (1H, t, J = 1.7 Hz, H-23), 7.41 (1H, br. t, J = 0.7 Hz, H-22); ^{13}C NMR (CDCl_3 , 125 MHz) δ 18.0 (C-30), 19.3 (C-2), 21.1 (C-18), 21.8 (C-28), 30.1 (C-29), 31.2 (C-12), 36.0 (C-2), 36.8 (C-6), 38.4 (C-13), 46.4 (C-10), 48.5 (C-9), 51.7 (C-8), 54.3 (C-15), 61.0 (C-5), 65.7 (C-19), 66.1 (C-14), 78.2 (C-17), 79.6 (C-1), 80.7 (C-4), 110.1 (C-20), 120.4 (C-21), 141.5 (C-22), 143.6 (C-23), 167.0 (C-16), 169.5 (C-3), 206.5 (C-7).

Methyl 4-hydroxybenzoate (4): colorless needle; mp 80-83°C; UV (CDCl_3) λ_{max} (log e) 206 (4.15), 255 (4.15) nm; IR (KBr) ν_{max} 3312, 3034, 1918, 1679, 1588 cm^{-1} ; ^1H NMR (CDCl_3 , 500 MHz) δ 3.92 (3H, s, 7-OCH₃), 6.91 (2H, d, J = 8.7 Hz, H-3 and H-5), 7.98 (2H, d, J = 8.7 Hz, H-2 and H-6); ^{13}C NMR (CDCl_3 , 125 MHz) δ 52.1 (7-OCH₃), 115.3 (C-3 and C-5), 122.3 (C-1), 132.0 (C-2 and C-6), 160.3 (C-4), 167.4 (C-7).

Syringin (5): colorless needle; mp 189-192°C; UV (CH_3OH) λ_{max} (log e) 219 (4.40), 265 (4.13) nm; IR (KBr) ν_{max} 3320, 3166, 1599, 1518, 985 cm^{-1} ; ^1H NMR (CD_3OD , 500 MHz) δ 3.23-3.20 (1H, m, H-3), 3.42 (2H, m, H-4 and H-5), 3.50-3.46 (1H, m, H-2), 3.67 (1H, dd, J = 12.0, 5.2 Hz, H-6), 3.78 (1H, dd, J = 12.0, 7.2 Hz, H-6), 3.86 (6H, s, H-3 and H-5), 4.22 (2H, dd, J = 5.6, 1.5 Hz, H-9), 4.86 (1H, d, J = 7.5 Hz, H-1), 6.32 (1H, d, J = 15.8 Hz, H-8), 6.54 (1H, d, J = 15.8 Hz, H-7), 6.75 (2H, s, H-2 and H-6); ^{13}C NMR (CD_3OD , 125 MHz) δ 57.1 (3-OCH₃, 5-OCH₃), 62.7 (C-6), 63.6 (C-9), 71.4 (C-4), 75.8 (C-2), 77.9 (C-5), 78.4 (C-3), 105.4 (C-1), 105.6 (C-2 and C-6), 130.1 (C-8), 131.3 (C-7), 135.3 (C-1), 136.0 (C-4), 154.4 (C-3 and C-5).

Results and Discussions

The MeOH extract obtained from the stem bark of *P. amurense* was fractionated into hexane, CHCl_3 , and BuOH

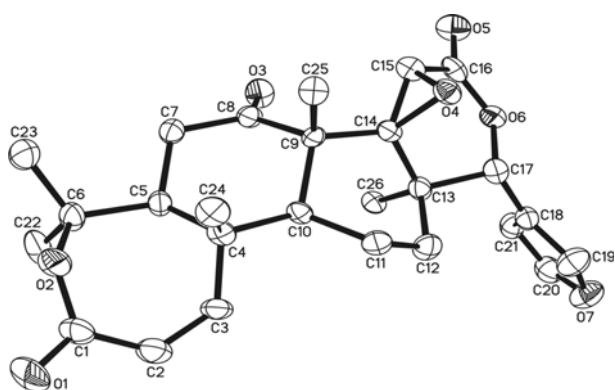


Fig. 3. ORTEO views of the X-ray molecular structures of **2**.

layers through solvent fractionation, and five compounds were isolated through the repeated chromatographic separation of CHCl_3 and BuOH fractions. Structural identifications of five compounds were carried out by interpretation of several spectral data, and comparison with the data described in the literature. Compounds **1-5** were identified as calodendrolide (**1**), obacunone (**2**), limonin (**3**), methyl 4-hydroxybenzoate (**4**), and syringin (**5**), among which calodendrolide (**1**) was first isolated from *P. amurense* in this study (Fig. 1 and 2), and structure **2** was verified by X-ray crystallographic analysis (Fig. 3). Compound **1** was obtained as an amorphous powder and, in EIMS, the molecular ion peak was observed at m/z 260. UV spectrum showed absorption maxima at 241 nm, and IR spectrum exhibited an absorption band at 1742 cm^{-1} ascribed to a carbonyl group. The ^1H NMR spectrum of **1** exhibited signals due to two tertiary methyl groups (δ 0.98 and 1.61), one olefinic proton (δ 5.99), one oxymethine proton (δ 5.52), and a β -substituted furan ring (δ 6.38, 7.41, and 7.42). In addition, ^{13}C -NMR and DEPT experiments indicated the presence of two methyls, two methylenes, six methines, and five quaternary carbons. On the basis of these spectral data, structure of **1** was assigned as calodendrolide (Fig. 4).¹¹⁻¹³ Compound **2** was obtained as colorless prisms and this compound was determined to be obacunone on the basis of the chemical and several NMR-spectroscopic studies, together with a comparison of the above data with those published in the literature,^{14,15} in addition, the relative stereochemistry of compound **2** was certified by X-ray crystallographic analysis (Fig. 3), which has not previously been reported.

Structure determination of **2** by X-ray diffraction.

Crystal data, $\text{C}_{26}\text{H}_{30}\text{O}_7$, $M = 454.51$, monoclinic, Space group, $P2(1)$, $a = 6.9802(12)$, $b = 13.986(3)$, $c = 13.309(2)$ Å, $\alpha = 90^\circ$, $\beta = 90.293(4)^\circ$, $\gamma = 90^\circ$, $V = 1299.3(4)$ Å³, $Z = 2$, $T = 173(2)$ K, $D_c = 1.310\text{ Mg/m}^3$, $F(000) = 548$, $\mu = 0.095\text{ mm}^{-1}$, $R1 = 0.0681$, $wR2 = 0.1613$, for 5474 [$R(\text{int}) = 0.0812$] independent reflections.

Compound **3** obtained as an amorphous powder, ^1H - and ^{13}C -NMR spectrum showed the similar limonoid skeleton as obacunone in B, C, D, and furan rings. Based on the results of 2D-NMR spectrum and HMBC experiments (Fig. 4), compound **3** was identified as limonin, together with a

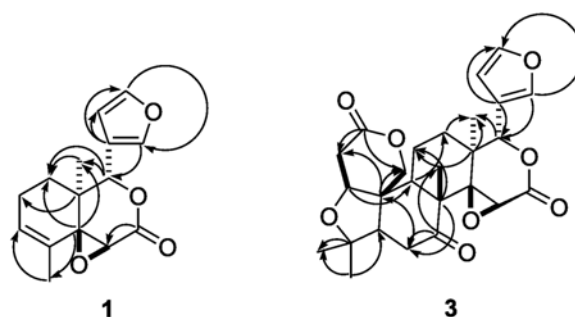


Fig. 4. Important HMBC correlations in compounds **1** and **3**.

comparison of the above data with those published in the literature.¹⁶⁻¹⁸

Compound **4** was obtained as a colorless prisms, and this compound was determined to be methyl 4-hydroxybenzoate on the basis of the chemical and ^1H - and ^{13}C -NMR spectrum, together with a comparison of the above data with those published in the literature.¹⁹ Compound **5** was obtained as a colorless needle, the comparison of several NMR and physical data on this compound with those of literatures^{20,21} led to identification as syringin.

Study of *P. amurense* led to the isolation of three limonoids, calodendrolide (**1**), obacunone (**2**), and limonin (**3**), and two phenolics, methyl 4-hydroxybenzoate (**4**) and syringin (**5**). Among the isolated compounds, calodendrolide (**1**) was for the first time isolated from this plant. In addition, structure of compound **2** was defined clearly with the aid of X-ray crystallographic analysis. The Rutaceae limonoids, obacunone (**2**) and limonin (**3**) present in large quantities in the byproducts of the citrus industry have previously been investigated for their insect antifeedant activities. Moreover, syringin (**5**) in phenolic compound has various pharmacological effects with little toxicity.

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