A Potent Anti-diabetic Agent from *Kalopanax pictus*

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To search for the anti-diabetic principle from the stem bark of *Kalopanax pictus*, seven kinds of chemical constituents including hederagenin glycosides and phenolic glycosides were isolated. The anti-diabetic evaluation of these isolates in the streptozotocin-induced diabetic rats exhibited that kalopanaxsaponin A has a potent anti-diabetic activity in contrast to a mild activity of hederagenin. In addition, significant hypocholesterolemic and hypolipidemic activities of kalopanaxsaponin A and hederagenin were observed. The structure-activity relationship of kalopanaxsaponin A was also investigated in the present work.

Key words: *Kalopanax pictus*, Araliaceae, Anti-diabetic, Kalopanaxsaponin A, Hederagenin

INTRODUCTION

The stem bark of *Kalopanax pictus* belonging to Araliaceae family has been used in traditional herbal medicine as tonics, analgesics and anti-diabetics. As the constituents of this stem bark, Sano *et al.* have isolated a number of hederagenin glycosides such as kalopanaxsaponin A-G in addition to phenolic glycosides such as liriodendrin, syringin and coniferaldehyde 4-O-glucoside as the constituents of this stem bark (Sano *et al.*, 1991). A number of saponin constituents having the aglycone moieties of hederagenin and 22α-hydroxyhederagenin have been isolated from the leaves of this plant (Shao *et al.*, 1989). The root of this plant contains saponin constituents having the aglycones of ursolic acid and hederagenin (Shao *et al.*, 1989). Similar saponins have been isolated from *Kalopanax pictum* var. magnificum, *Kalopanax pictum* var. typicum and *Kalopanax pictum* var. chinense (Park *et al.*, 1991, Cho, *et al.*, 1991, Lee *et al.*, 1991). Lee *et al.* (1995) have reported the presence of saturated fatty acid and linoleic acid from the stem bark of *K. pictus*.

Although a number of chemical constituents of this plant have been identified, detailed pharmacological activities remains mostly unknown except antihypertensive activity and analgesic activity of liriodendrin of this plant (Lee *et al.*, 1995). In the present study, we isolated main chemical constituents such as saponins together with phenolic glycosides from the stem bark of *Kalopanax pictus* and identified their structures. Anti-diabetic activities of these constituents were evaluated in the streptozotocin-induced diabetic rats.

Kalopanaxsaponin A was found to considerably improve diabetic symptoms at the low dose of 10 mg/kg (i.p.).

MATERIALS AND METHODS

Instruments and reagents

Melting points were determined on a Electrothermal digital melting point apparatus (Electrothermal, USA) and are uncorrected. Optical rotations were measured on a JASCO DIP-360 digital polarimeter at 25°C. IR spectra were recorded on a Bomem MB-100 FT-IR spectrometer in KBr disks. ¹H- and ¹³C-NMR spectra were taken on a Brucker-AM 500 with TMS as an internal standard. Olea-nolic acid employed in the biological test is authentic specimen.

Plant material

The stem bark of *Kalopanax pictus* was collected in August 1995 in Kangwon province, Korea, and the plant was identified by Prof. S.Y. Yun(Department of Botanical Resources, Sangji University, Wonju, Korea). A voucher specimen was deposited in the herbarium of Life Science and Natural Resources, Sangji University, Wonju, Korea.

Extraction and isolation

Dried stem bark (9.6 kg) of *Kalopanax pictus* was pulverized and extracted three times with methanol.
under reflux. The methanolic extract was filtered and evaporated on a rotary evaporator under reduced pressure to obtain a viscous mass (1.28 kg) of MeOH extract. This material was suspended in H2O and then partitioned with CHCl3, EtOAc, and n-BuOH to give a CHCl3 soluble fraction (435 g), an EtOAc soluble fraction (67 g), and an n-BuOH soluble fraction (450 g).

A part of EtOAc-soluble fraction (20 g) was further fractionated by use of silica gel (Merck Art 7734, Germany) column chromatography using CHCl3-MeOH-H2O (7:3:1, lower phase). Each subfraction was successively purified by the method of Sephadex LH-20 and/or ODS column chromatography and then recrystallized with methanol solvent to give compound 1, 2, 3 and 4, respectively.

By the similar procedures, a part of n-BuOH fraction (20 g) was further fractionated by use of silica gel column chromatography with the eluting solvent of CHCl3-MeOH-H2O (65:35:10, lower phase). Each subfraction was successively purified by Sephadex LH-20 and/or ODS column chromatography followed by recrystalization to give compound 5, 6 and 7, respectively.

The mp, [α]D, and 1H- and 13C-NMR spectral data of all of the isolated compounds above were measured and identified as follows: 1 (coniferylaldehyde 4-O-glucoside), 2 (kalopanaxsaponin A), 3 (syringin), 4 (3-0-[xylopyranosyl(1→3)-o-R-rhamnopyranosyl(1→2)-o-L-arabino-pyranosyl]-hederagenin), 5 (liriodendrin), 6 (kalopanaxsaponin B), 7 (3-O-[β-D-glucopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl]-hederagenin 28-O-[α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl]).

**Compound 1:** Colorless needles from MeOH, mp 209-211°C, [α]D -39.3° (c=0.3, pyridine). Compound 1 was characterized as coniferylaldehyde 4-O-glucoside by co-TLC, mmp and [α]D with authentic specimen.

**Compound 2:** Colorless needles from MeOH, mp 265-268°C (dec.), [α]D +18° (MeOH, c=0.30), IR v(KBr) cm⁻¹: 3415 (broad, OH), 1698 (COOH), 1058 (glycoside); 1H-NMR (300 MHz, pyridine-d₅) δ: see Table I; 13C-NMR (300 MHz, pyridine-d₅) δ: see Table II.

**Compound 3:** Colorless needles from H2O, mp 192-193°C, [α]D -21.4° (c=1.3, MeOH). Compound 3 was characterized as syringin by comparison of co-TLC, mmp and [α]D with authentic specimen.

**Compound 4:** Colorless needles from MeOH, mp 218-220°C (dec.), [α]D +11° (MeOH, c=0.28), IR v(KBr) cm⁻¹: 3417 (broad, OH), 1695 (COOH), 1050 (glycoside); 1H-NMR (300 MHz, pyridine-d₅) δ: see Table I, 13C-NMR (300 MHz, pyridine-d₅) δ: see Table II.

**Compound 5:** Colorless needles from H2O, mp 255°C, The signals of 13C-NMR spectrum resemble those reported for (+)-syringaresinol di-O-β-D-glucopyranoside (Higuchi et al., 1976). Compound 5 was characterized as a mixture of di-O-β-D-glucopyranosides of (+) and (-)-syringaresinol according to those reported from Kalopanax pictus.

**Compound 6:** Amorphous powder, mp 204-212°C (dec.), [α]D -18° (MeOH, c=0.69), IR v(KBr) cm⁻¹: 3416 (broad, OH), 1728 (COO⁻), 1054 (glycoside); 1H-NMR (300 MHz, pyridine-d₅) δ: see Table I; 13C-NMR (300 MHz, pyridine-d₅): see Table II.

**Compound 7:** Amorphous powder, mp 212-217°C (dec.), [α]D -25° (MeOH, c=0.96), IR (KBr) cm⁻¹: 3417 (broad, OH), 1729 (COO⁻), 1052 (glycoside); 1H-NMR (300 MHz, pyridine-d₅) δ: see Table I; 13C-NMR (300 MHz, pyridine-d₅) δ: see Table II.

**Preparation of hederagenin**

Complete acid hydrolysis of n-BuOH fraction was