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# 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, Hedearagenin

## 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 coniferylaldehyde 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 antihepatotoxic 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 contituents 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.).

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## 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. $^{1}$H- and $^{13}$C-NMR spectra were taken on a Brucker-AM 500 with TMS as an internal standard. Oleanolic 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

**Table I.** $^1$H-NMR signals of compound **2**, **4**, **6** and **7**

| | **2** | **4** | **6** | **7** |
|---|---|---|---|---|
| Aglycone | | | | |
| H-12 | 5.48 (1H, brs) | 5.45 (1H, brs) | 5.35 (1H, brs) | 5.38 (1H, brs) |
| 24 | 1.02 (3H, s) | 1.13 (1H, s) | 0.91 (3H, s) | 0.96 (3H, s) |
| 25 | 0.90 (3H, s) | 0.92 (3H, s) | 0.91 (3H, s) | 1.08 (3H, s) |
| 26 | 0.98 (3H, s) | 0.97 (3H, s) | 1.03 (3H, s) | 1.08 (3H, s) |
| 27 | 1.20 (3H, s) | 1.23 (3H, s) | 1.12 (3H, s) | 1.17 (3H, s) |
| 29 | 0.90 (3H, s) | 0.92 (3H, s) | 0.83 (3H, s)[a] | 0.85 (3H, s)[a] |
| 30 | 0.97 (3H, s) | 0.98 (3H, s) | 0.82 (3H, s)[a] | 0.83 (3H, s)[a] |
| 3-position | 5.07 (1H, d, 6.0b) | | | |
| H-1 (ara) | | 5.03 (1H, d, 6.0) | 5.04 (1H, d, 6.0) | 5.04 (1H, d, 6.0) |
| 1 (rha) | | 6.08 (1H, brs) | 6.00 (1H, brs) | 6.21 (1H, brs) |
| 1 (xyl) | | 5.36 (1H, d, 7.0) | | 5.2 (1H, d, 7.5) |
| 28-position | | | | |
| H-1 (glc) | | | 6.09 (1H, d, 8.1) | 6.17 (1H, d, 8.1) |
| 1 (glc) | | | 4.88 (1H, d, 8.0) | 4.94 (1H, d, 8.0) |
| 1 (rha) | | | 5.77 (1H, brs) | 5.75 (1H, brs) |

[a]Values may be interchanged in each column.
[b]Values represent coupling constants (Hz)

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 $H_2O$ and then partitioned with $CHCl_3$, EtOAc, and *n*-BuOH to give a $CHCl_3$ soluble fraction (435 g), an EtOAc soluble fraction (67 g), and a *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 $CHCl_3$-MeOH-$H_2O$ (7:3:1, lower phase). Each subfraction was successively purified by the method of Sephadex LH-20 and/or ODS column chromatography and then recrystalized 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 $CHCl_3$-MeOH-$H_2O$ (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, $[\alpha]_D$, and $^1$H- and $^{13}$C-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-*O*-[xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabino-pyran-osyl]-hederagenin), **5** (liriodendrin), **6** (kalopanaxsaponin B), **7** (3-*O*-[β-D-xylopyranosyl(1→3)-α-L-rhamnopyra-nosyl(1→2)-α-L-arabinopyranosyl]-hederagenin 28-*O*-[α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside].

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

**Compound 2:** Colorless needles from MeOH, mp 265~268°C (dec.), $[\alpha]_D$ +18° (MeOH, c=0.30), IR ν(KBr) cm$^{-1}$: 3415 (broad, OH), 1698 (COOH), 1058 (gly- co-side); $^1$H-NMR (300 MHz, pyridine-$d_5$) δ: see Table I; $^{13}$C-NMR (300 MHz, pyridine-$d_5$) δ: see Table II.

**Compound 3:** Cololess needles from $H_2O$, mp 192~193°C, $[\alpha]_D$ -21.4° (c=1.3, MeOH). Compound 3 was charaterized as syringin by comparison of co-TLC, mmp and $[\alpha]_D$ with authentic specimen.

**Compound 4:** Colorless needles from MeOH, mp 218~220°C (dec.), $[\alpha]_D$ +11° (MeOH, c=0.28), IR ν(KBr) cm$^{-1}$: 3417 (broad, OH), 1695 (COOH), 1050 (gly- co-side); $^1$H-NMR (300 MHz, pyridine-$d_5$) δ: see Table I, $^{13}$C-NMR (300 MHz, pyridine-$d_5$) δ: see Table II.

**Compound 5:** Colorless needles from $H_2O$, mp 255 °C, The signals of $^{13}$C-NMR spectrum resemble those reported for (+)-syringaresinol di-*O*-β-Dglucopyranoside (Higuchi *et al.*, 1976). Compound 5 was charaterized as a mixture of di-*O*-β-D-glucopyranosides of (+)- and (-)-syringaresinol according to those reported from *Kalopanax pictus*.

**Compound 6:** Amophous powder, mp 204~212°C (dec.), $[\alpha]_D$ -18° (MeOH, c=0.69), IR ν(KBr) cm$^{-1}$: 3416 (broad, OH), 1728 (COO-), 1054 (glycoside); $^1$H-NMR (300 MHz, pyridine-$d_5$) δ: see Table I; $^{13}$C-NMR (300 MHz, pyridine-$d_5$): see Table II.

**Compound 7:** Amorphous powder, mp 212~217°C (dec.), $[\alpha]_D$ -25° (MeOH, c=0.96), IR (KBr) cm$^{-1}$: 3417 (broad, OH), 1729 (COO-), 1052 (glycoside); $^1$H-NMR (300 MHz, pyridine-$d_5$) δ: see Table I; $^{13}$C-NMR (300 MHz, pyridine-$d_5$) δ: see Table II.

### Preparation of hederagenin

Complete acid hydrolysis of *n*-BuOH fraction was

**Table II.** $^{13}$C-NMR signals of compound **2**, **4**, **6** and **7**

| | 2 | 4 | 6 | 7 | | 2 | 4 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|---|
| Aglycon | | | | | Sugars at C-3 of aglycon | | | | |
| C-1 | 38.9 | 38.9 | 38.9 | 38.9 | Ara-3 | 74.6 | 75.6 | 73.8 | 74.3 |
| 2 | 26.1 | 26.1 | 26.0 | 25.9 | 4 | 69.2 | 69.5 | 69.0 | 69.3 |
| 3 | 81.0 | 81.0 | 81.0 | 82.6 | 5 | 65.6 | 66.3 | 64.9 | 66.2 |
| 4 | 43.4 | 43.5 | 43.3 | 43.4 | Rha-1 | 101.6 | 101.3 | 101.4 | 101.0 |
| 5 | 47.7 | 47.6 | 47.5 | 47.5 | 2 | 72.3 | 71.9 | 72.0[a] | 71.9 |
| 6 | 18.1 | 18.0 | 18.0 | 18.3 | 3 | 72.5 | 83.0 | 72.5[a] | 82.7 |
| 7 | 32.8 | 32.8 | 32.4 | 32.4 | 4 | 74.0 | 72.9 | 73.8 | 72.5 |
| 8 | 39.7 | 39.7 | 39.8 | 39.8 | 5 | 69.6 | 69.7 | 69.6 | 69.6 |
| 9 | 48.1 | 48.1 | 48.0 | 48.1 | 6 | 18.5 | 18.4 | 18.3 | 18.2 |
| 10 | 36.8 | 36.8 | 36.7 | 36.8 | Xyl-1 | | 107.6 | | 107.1 |
| 11 | 23.7 | 23.7 | 23.6 | 23.7 | 2 | | 75.3 | | 75.2 |
| 12 | 122.5 | 122.5 | 122.7 | 122.7 | 3 | | 78.4 | | 77.0 |
| 13 | 144.8 | 144.7 | 144.0 | 144.0 | 4 | | 71.1 | | 70.7 |
| 14 | 42.1 | 42.0 | 42.0 | 42.0 | 5 | | 67.4 | | 67.1 |
| 15 | 28.3 | 28.2 | 28.1 | 28.2 | Sugars at C-28 of aglycon | | | | |
| 16 | 23.8 | 23.7 | 23.2 | 23.2 | Glc-1 | | | 95.4 | 95.5 |
| 17 | 46.6 | 46.5 | 46.1 | 46.1 | 2 | | | 75.0 | 75.0 |
| 18 | 41.9 | 41.9 | 41.5 | 41.5 | 3 | | | 78.4 | 78.4 |
| 19 | 46.4 | 46.5 | 46.1 | 46.1 | 4 | | | 70.1[b] | 70.5[b] |
| 20 | 30.9 | 30.9 | 30.6 | 30.6 | 5 | | | 76.3 | 76.2 |
| 21 | 34.2 | 34.1 | 33.9 | 33.9 | 6 | | | 70.6 | 71.0 |
| 22 | 33.2 | 33.2 | 32.6 | 32.6 | Glc-1 | | | 104.4 | 104.5 |
| 23 | 63.9 | 63.9 | 63.9 | 63.9 | 2 | | | 73.6 | 73.6 |
| 24 | 13.9 | 14.1 | 13.7 | 14.0 | 3 | | | 77.8 | 77.8 |
| 25 | 16.0 | 16.0 | 16.0 | 16.1 | 4 | | | 78.4 | 78.2 |
| 26 | 17.4 | 17.4 | 17.4 | 17.4 | 5 | | | 76.8 | 76.9 |
| 27 | 26.1 | 26.1 | 25.9 | 25.9 | 6 | | | 61.2 | 61.0 |
| 28 | 180.3 | 180.1 | 176.5 | 176.5 | Rha-1 | | | 102.5 | 102.5 |
| 29 | 33.2 | 33.2 | 33.0 | 33.0 | 2 | | | 72.2[a] | 72.1[a] |
| 30 | 23.6 | 23.7 | 23.7 | 23.7 | 3 | | | 72.3[a] | 72.3[a] |
| Sugars at C-3 of aglycon | | | | | 4 | | | 73.7 | 73.5 |
| Ara-1 | 104.3 | 104.6 | 103.8 | 104.3 | 5 | | | 69.0[b] | 69.1[b] |
| 2 | 75.7 | 75.0 | 75.8 | 75.4 | 6 | | | 18.3 | 18.2 |

[a,b]Values may be interchanged in each column.

carried out by 5%-HCl under reflux for 5 hours. The reactant was patitioned between water and ethylacetate. Ethyl acetate soluble fraction was subjected to chromatographed on Si gel column with eluting solvent of $CHCl_3$-MeOH-$H_2O$ (25:8:5, lower phase). Hederagenin-contained subfraction was recrystalized in methanol.

## Hederagenin

Colorless needles from MeOH, mp 318~320°C, $[\alpha]_D$ +81° (c=0.1, pyridine); IR, $\nu_{max}$ (KBr) 3440, 1703, 1039, 1025, 824, 811 cm$^{-1}$; $^1$H-NMR (pyridine-$d_5$) δ: 0.93 (3H, s, $CH_3$), 0.99 (6H, s, 2×$CH_3$), 1.04 (6H, s, 2×$CH_3$), 1.23 (3H, s, $CH_3$), 3.31 (1H, dd-like, H-18), 5.49 (1H, brs, H-12); $^{13}$C-NMR (300 MHz, pyridine-$d_5$): hederagenin (C-1-30)-38.9, 27.6, 73.7, 42.9, 48.8, 18.7, 33.0, 39.8, 48.2, 37.3, 23.8, 122.7, 145.0, 42.2, 28.4, 23.8, 46.7, 42.0, 46.5, 31.0, 34.3, 33.3, 68.2, 13.1, 16.0, 17.5, 26.2, 180.4, 33.3, 23.8; EIMS, m/z (rel. int., %) 472[$M^+$] (0.8), 454[M-$H_2O$]$^+$ (1.0), 248 (59.9).

## Experimental animal

Male Sprague-Dawley rats were fed commercial standard rat diet and water at *libitum.*, and maintained at 20±2°C and with the illumination of a 12 h light/dark cycle.

## Induction of diabetic rats and administration of isolated compounds

Diabetes was induced with streptozotocin (50 mg/kg, intravenously in 0.01 M citrate buffer, pH 4.5). The rats were considered diabetic only if their blood glucose concentration exceeded 300 mg/dl as measured with a comercially available One Touch II (Lifescan) blood glucose strips. Animals were diabetic at least 7 days prior to use. Samples (25 mg/kg or 50 mg/kg in 0.9% saline) were given for 3 days by intraperitoneal injection.

### Determination of serum glucose, cholesterol, triglyceride, lipoprotein cholesterols, total lipid and triglyceride

Serum glucose levels were measured with a commercially available One Touch II (Lifescan) blood glucose strips test. Aliquots of serum were assayed enzymatically for total cholesterol (Rudel *et al.*, 1973), lipoprotein-cholesterol (Noma *et al.*, 1978), total lipid (Epstein *et al.*, 1972) and triglyceride (McGown *et al.*, 1983).

### Statistical analysis

Data were summarized as mean±S.D. We used Duncan's new multiple range test to determine the statistical significance.

## RESULTS AND DISCUSSION

Isolated compounds **1-7** were identified to be coniferylaldehyde 4-*O*-glucoside (**1**), kalopanaxsaponin A (**2**), syringin (**3**), 3-[*O*-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl]-hederagenin (**4**), liriodendrin (**5**), kalopanaxsaponin B(**6**), 3-*O*-β-D-xylopyranosyl(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-glucopyranoside] (**7**) by comparisons of physicochemical constants and spectral data with literature data (Sano *et al.*, 1991). Porzel *et al.* have reported the isolation of **7** for the first time from *Kalopanax septemlobus* (Porzel *et al.*, 1992). **7** was named as kalopanaxsaponin H in the present paper. However, **4** was not employed in the present biological test. All the structures of isolated saponins were clarified by showing the assignment of $^{1}$H- and $^{13}$C-NMR spectral data in Table I and Table II.

These isolated compounds (**1**, **2**, **3**, **5**, **6**, **7**) were intraperitoneally administered to streptozotocin-induced diabetic rats once a day for three days. The blood was collected 24 hours after the final dose. From the serums of these blood, serum glucose level, total cholesterol, HDL-cholesterol, (VLDL+LDL)-cholesterol, total lipid and triglyceride were measured.

From these results, it was revealed that kalopanaxsaponin A have a very strong anti-diabetic action in contrast to a mild anti-diabetic action of hederagenin. This compound decreased the serum glucose level, by 93% with 25 mg/kg dose as shown in Table I. Other compounds did not show this activity at all.

First of all, as hypoglycemic activity of kalpanaxsaponin A, this compound decreased the serum glucose level which was tremendously elevated by streptozotocin administration, by 93% with 25 mg/kg dose, as

**Table III.** Effect of the constituents of the stem bark of *Kalopanax pictus* on the serum glucose levels in normal and diabetic rats

| Group | dose (mg/kg) | Glucose (mg/dl) |
|---|---|---|
| Normal | | 88.5±10.79$^{a}$ |
| Streptozotocin | | 323.3±52.52$^{b}$ |
| Oleanolic acid | 50 | 316.3±18.29$^{b}$ |
| Hederagenin | 50 | 203.3±45.71$^{c}$ |
| Kalopanaxsaponin A | 25 | 105.8±12.23$^{a}$ |
| Kalopanaxsaponin B | 50 | 315.0±49.52$^{b}$ |
| Kalopanaxsaponin H | 25 | 320.8±54.21$^{b}$ |
| CFA | 25 | 325.9±48.6$^{b}$ |
| Syringin | 25 | 330.2±41.0$^{b}$ |
| Liriodendrin | 50 | 329.0±56.11$^{b}$ |

Abbreviation, CFA: coniferylaldehyde 4-*O*-glucoside; Samples were intraperitoneally administered daily for three days in streptozotocin-induced diabetic rats, and decapitated, 24 hrs after the final dose of sample. The assay procedure is described in the experimental methods. Values are mean±S.D. (n=8). Values with the same letter are not significantly different at $p<0.05$., in Duncan's new multiple range test.

**Table IV.** Effect of the constituents of the stem bark of *Kalopanax pictus* on serum cholesterol and lipoprotein levels in normal and diabetic rats

| Group | dose (mg/kg) | cholesterol (mg/dl) | | |
|---|---|---|---|---|
| | | Total | HDL | VLDL+LDL |
| Normal | | 86.9±8.21$^{a}$ | 30.9±4.42$^{a}$ | 58.9±6.74$^{a}$ |
| Streptozotocin | | 127.5±7.69$^{b}$ | 21.9±2.54$^{b,c}$ | 105.2±12.1$^{b,c}$ |
| Oleanolic acid | 50 | 121.3±9.60$^{b,c}$ | 21.1±3.04$^{c}$ | 105.6±12.1$^{b,c}$ |
| Hederagenin | 50 | 107.2±9.35$^{c,d}$ | 25.4±3.34$^{b,c}$ | 88.5±10.9$^{c,d}$ |
| Kalopanaxsaponin A | 25 | 104.1±6.69$^{d}$ | 26.3±2.69$^{a,b}$ | 69.4±15.3$^{a,d}$ |
| Kalopanaxsaponin B | 50 | 117.4±17.5$^{b,c,d}$ | 20.8±4.47$^{c}$ | 110.7±14.0$^{b}$ |
| Kalopanaxsaponin H | 25 | 116.9±15.3$^{b,c,d}$ | 21.7±2.99$^{b,c}$ | 108.0±17.1$^{b}$ |
| CFA | 25 | 122.5±13.9$^{b,c,d}$ | 20.5±2.81$^{b,c}$ | 110.1±7.90$^{b}$ |
| Syringin | 25 | 124.0±15.2$^{b,c,d}$ | 21.6±2.55$^{b,c}$ | 109.5±6.89$^{b}$ |
| Liriodendrin | 50 | 120.8±14.4$^{b,c,d}$ | 20.6±2.54$^{b,c}$ | 112.1±8.62$^{b}$ |

Abbreviation, CFA: coniferylaldehyde 4-O-glucoside; Samples were intraperitoneally administered daily for three days in streptozotocin-induced diabetic rats, and decapitated, 24 hrs after the final dose of sample. The assay procedure is described in the experimental methods. Values are mean±S.D. (n=8). Values with the same letter are not significantly different at $p<0.05$., in Duncan's new multiple range test.

**Table V.** Effect of the constituents of the stem bark of *Kalopanax pictus* on serum total lipid and triglyceride levels in normal and diabetic rats

| Group | dose (mg/kg) | total lipid (mg/dl) | triglyceride (mg/dl) |
|---|---|---|---|
| Normal | | $251.6\pm26.46^{a}$ | $74.7\pm12.88^{a}$ |
| Streptozotocin | | $341.4\pm44.29^{b,c}$ | $132.2\pm20.33^{b}$ |
| Oleanolic acid | 50 | $320.9\pm28.21^{b,c,d}$ | $121.0\pm14.62^{b,c}$ |
| Hederagenin | 50 | $304.6\pm18.31^{b,c}$ | $107.1\pm10.84^{c,d}$ |
| Kalopanaxsaponin A | 25 | $289.2\pm13.39^{a,d}$ | $99.0\pm10.03^{d}$ |
| Kalopanaxsaponin B | 50 | $317.9\pm17.85^{b,c,d}$ | $128.1\pm17.38^{b,c}$ |
| Kalopanaxsaponin H | 25 | $339.6\pm38.74^{b,c}$ | $119.8\pm13.25^{b,c,d}$ |
| CFA | 25 | $356.3\pm31.28^{b}$ | $126.8\pm13.52^{b}$ |
| Syringin | 25 | $349.5\pm29.82^{b}$ | $124.5\pm12.9^{b}$ |
| Liriodendrin | 50 | $352.1\pm48.71^{b}$ | $129.4\pm14.07^{b}$ |

Abbreviation, CFA: coniferylaldehyde 4-*O*-glucoside Samples were intraperitoneally administered daily for three days in streptozotocin-induced diabetic rats, and decapitated 24 hrs after the final dose. The assay procedure is described in the experimental methods. Values are mean±S.D. (n=8). Values with the same letter are not significantly different at $p<0.05$, in Duncan's new multiple range test.

shown in Table I. Meanwhile, as shown in Table II, this compound significantly decreased the values of serum total cholesterol, and serum (VLDL+LDL)-cholesterol which were elevated by the induction of *diabetes-mellitus*, by 41% and 73%, respectively, while enhanced the HDL-cholesterol level by 49%. These effects suggested that kalopanaxsaponin A improved hypercholesterolemia induced in experimental diabetes. Furthermore, as shown in Table III, total lipid and triglyceride levels were decreased by 58% and 57%, respectively. These findings indicated that the hyperlipidemia elicited by *diabetes-mellitus* was cosiderably reduced.

Besides kalopanaxsaponin A, hederagenin decreased serum glucose level by 51% with 50 mg/kg (i.p.) dose (Table I). The hypoglycemic effect of hederagenin was significantly lower than that of kalopanaxsaponin A. In addition, hederagenin decreased the levels of total cholesterol and (VLDL+LDL)-cholesterol by 50% and 36%, respectively. However, this compound considerably elevated the value of serum HDL-cholesterol by 39% (Table II). Furthermore, the compound of hederagenin decreased the levels of total lipid and triglyceride by 41% and 44%, respectively (Table III). Its hypolipidemic activity is lower than that of kalopanxsaponin A(**2**).

As shown in Fig. 1, the hypoglycemic effects of **2** were not significantly different over the range of 10~50 mg/kg dose. The change of hypoglycemic- and hypolipidemic effects of **2** were not also observed in the same dose ranges (Fig. 1 and 2). Thus, the minimal effective dose in this test is 10 mg/kg. Administration of hederagenin with 25 mg/kg and 50 mg/kg showed similar activity values each other in these biological activity tests. However, administration of 10 mg/kg of hederagenin showed weaker activity compared 25 mg/kg and 50 mg/kg doses. In addition, the reduction of blood lipid, cholesterol and lipoproteins by **2** and its aglycone might result from increased blood-glucose

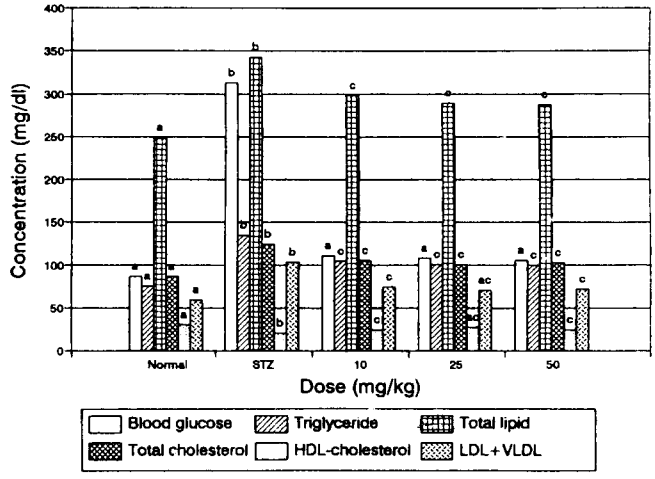


**Fig. 1.** Dose responses of streptozotocin-induced rats to Kalopanaxsapoin A in the concenteration of blood glucose, triglyceride, total lipid, total cholesterol, HDL-cholesterol, and LDL+VLDL. The values with different letters at each dosage differ significantly at the 0.05 probability level.

metabolism.

Based on the above findings it can be concluded that **2** exhibits a strong anti-diabetic activity whereas hederagenin, the aglycone of **2**, exhibits a significantly lower activity than **2**. Furthermore, though significant differences between the activity values of **2** and hederagenin exist, it was considered that hederagenin and 2 share a similar anti-diabetic mechanism, based on all the biological data described above. Therefore, it was estimated that the important active moiety is hederagenin of the aglycone and further sugar attachment to C-3 considerably potentiate the activity.

On the other hand, oleanolic acid, 23-hydroxyl reductant of hederagenin, did not show anti-diabetic activity at all. Therefore, there is possibility that 23-hydroxyl functionality of hederagenin play a key role in this action. In addition, **6** and **7**, which have este-

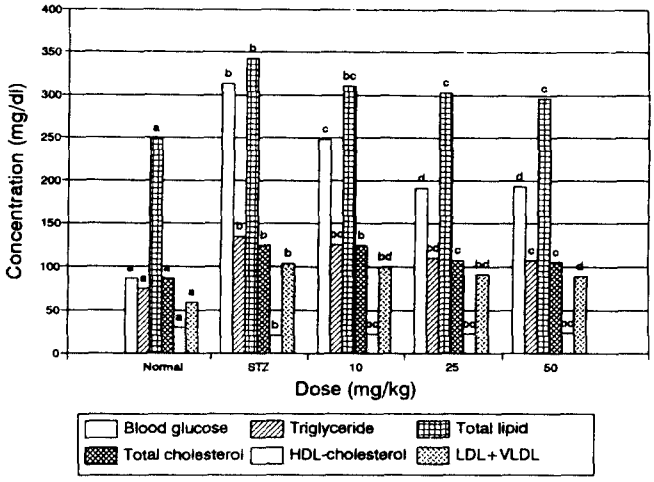


**Fig. 2.** Dose responses of streptozotocin-induced rats to hederagenin in the concenteration of blood glucose, triglyceride, total lipid, total cholesterol, HDL-cholesterol, and (LDL+VLDL)-cholesterol. The values with different letters at each dosage differ significantly at the 0.05 probability level.

rified sugar moieties attached 17-carboxyl, did not exhibit anti-diabetic action. Therefore, this fact imply that the presence of 17-carboxyl of hederagenin is essential for anti-diabetic action. From the comparisons between these compounds of hederagenin analogues, it was considered that the introduction of polar moieties such as sugars to 3-OH of hederagenin should result in profound influences on the anti-diabetic values. Although **6** and **7** did not exhibit any anti-diabetic activity, it was considered that oral administration of these compound might exert anti-diabetic action through biotransformations by intestinal microorganisms of these glycosides to their metabolite responsible.

On the other hand, natural aromatic compounds of this plant material such as coniferylaldehyde 4-*O*-glucoside, syringin and liriodendrin did not improve *diabetes-mellitus* induced by streptozotocin. Therefore, it was unambiguously suggested that pentacyclic triterpenoids such as hederagenin derivatives is responsible for anti-diabetic activity.

In addition, it has been reported that triterpenoids such as the derivatives of tormentic acid (Villar *et al.*, 1985), pomolic acid (Norra *et al.*, 1988) and polyhydroxylated triterpenoids (Thomas *et al.*, 1991) exhibit hypoglycemic activities. However, this is the first report that kalopanaxsaponin A exhibit a very strong activity.

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