# Metabolism of Kalopanaxsaponin B and H by Human Intestinal Bacteria and Antidiabetic Activity of Their Metabolites

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**To investigate the relationship between the intestinal bacterial metabolism of kalopanaxsaponin B and H from *Kalopanax pictus* (Araliaceae), and their antidiabetic effect, kalopanaxsaponin B and H were metabolized by human intestinal microflora and the antidiabetic activity of their metabolites was measured. Human intestinal microflora metabolized kalopanaxsaponin B to kalopanaxsaponin A, hederagenin 3-*O*-α-L-arabinopyranoside and hederagenin. The main metabolites of kalopanaxsaponin B were kalopanaxsaponin A and hederagenin. Kalopanaxsaponin H was metabolized to kalopanaxsaponin A and I, hederagenin 3-*O*-α-L-arabinopyranoside and hederagenin. The main metabolites of kalopanaxsaponin H were kalopanaxsaponin I and hederagenin. Among kalopanaxsaponin B, H and their metabolites, kalopanaxsaponin A showed the most potent antidiabetic activity, followed by hederagenin. However, the main components, kalopanaxsaponin B and H, in *K. pictus* were inactive.**

**Key words** antidiabetic activity; kalopanaxsaponin B; kalopanaxsaponin H; kalopanaxsaponin A; intestinal bacteria; *Kalopanax pictus* (Araliaceae)

In traditional medicines, most herbal drugs are administered orally as decoctions. Active components of these herbal drugs are inevitably brought into contact with intestinal microflora in the alimentary tract. Some could be transformed by the intestinal bacteria before absorption from the gastrointestinal tract. Studies on the metabolism of the components of these drugs by human intestinal microflora are of a great importance to an understanding of their biological effects.[1)]

In Korea, the stem bark of *Kalopanax pictus* belonging to the Araliaceae family has been used in tonic, analgesic, and antidiabetic preparations. From the stem bark of this plant, Sano *et al.* have isolated hederagenin glycosides (kalopanaxsaponin A—G), liriodendrin, sinigrin and coniferin.[2)] Shao *et al.* reported saponins having hederagenin and 22α-hydroxyhederagenin as aglycones from the leaves of this plant.[3,4)] The biological activity of this plant has not been studied apart from the antihepatotoxic activity and analgesic activity of liriodendrin. Therfore, we could not obtain any information about the metabolism of kalopanaxsaponin B (KPB) and kalopanaxsaponin H (KPH), the main components of *K. pictus* by human intestinal bacteria or their biological activity. Furthermore, these glycosides are not easily the absorbed intact from the gastrointestinal tract. In order to understand the nature of the metabolites of KPH and KPH produced by human intestinal bacteria and how their biological activity changes, we investigated the metabolism of KPB and KPH by human intestinal bacteria and the antidiabetic activity of their metabolites.

## MATERIALS AND METHODS

**Instrument** Melting points were determined on an Electrothermal digital melting point apparatus. 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. The TLC chromatogram of metabolites was quantita-

Table 1. $^{1}$H-NMR Chemical Shifts of Kalopanaxsaponin B, H and Their Metabolites

| Position | Chemical shifts (ppm) HA | KPA | KPI | KPB | KPH |
|---|---|---|---|---|---|
| Aglycone | | | | | |
| H-12 | 5.47 (1H, br s) | 5.48 (1H, br s) | 5.45 (1H, br s) | 5.35 (1H, br s) | 5.38 (1H, br s) |
| 24 | 1.01 (3H, s) | 1.02 (3H, s) | 1.13 (3H, s) | 0.91 (3H, s) | 0.96 (3H, s) |
| 25 | 0.92 (3H, s) | 0.90 (3H, s) | 0.92 (3H, s) | 0.91 (3H, s) | 1.08 (3H, s) |
| 26 | 0.98 (3H, s) | 0.98 (3H, s) | 0.97 (3H, s) | 1.03 (3H, s) | 1.08 (3H, s) |
| 27 | 1.22 (3H, s) | 1.20 (3H, s) | 1.23 (3H, s) | 1.12 (3H, s) | 1.17 (3H, s) |
| 29 | 0.92 (3H, s) | 0.90 (3H, s) | 0.92 (3H, s) | 0.83 (3H, s) | 0.85 (3H, s) |
| 30 | 0.98 (3H, s) | 0.97 (3H, s) | 0.98 (3H, s) | 0.82 (3H, s) | 0.83 (3H, s) |
| 3-Position | | | | | |
| H-1(ara) | 4.97 (1H, d, $J$=7.6 Hz) | 5.07 (1H, $J$=6.0 Hz) | 5.03 (1H, d, $J$=6.0 Hz) | 5.04 (1H, d, $J$=6.0 Hz) | 5.04 (1H, d, $J$=6.0 Hz) |
| 1(rha) | | 6.18 (1H, br s) | 6.08 (1H, br s) | 6.00 (1H, br s) | 6.21 (1H, br s) |
| 1(xyl) | | | 5.36 (1H, d, $J$=7.0 Hz) | | 5.26 (1H, br s) |
| 28-Position | | | | | |
| H-1(Glc) | | | | 6.09 (1H, d, $J$=8.1 Hz) | 6.17 (1H, d, $J$=8.1 Hz) |
| 1(Glc) | | | | 4.88 (1H, d, $J$=8.0 Hz) | 4.94 (1H, d, $J$=8.0 Hz) |
| 1(rha) | | | | 5.77 (1H, br s) | 5.75 (1H, br s) |

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tively analyzed with a Shimazu CS-920 TLC-scanner.

**Materials** Oleanic acid and streptozotocin were purchased from Sigma Chem. Co. (U.S.A.) and general anaerobic medium (GAM) and blood liver agar medium (BL) were from Nissui Pharm. Co., Ltd. (Japan).

**Thin-Layer Chromatography** TLC for KPH (kalopanaxsaponin H), KPB (kalopanaxsaponin B), KPA (kalopanaxsaponin A), KPI (kalopanaxsaponin I), HA (hederagenin 3-*O*-$\alpha$-L-arabinopyranoside) and hederagenin was performed on silica-gel plates (Merk, Silica-gel $60F_{254}$) with $CHCl_3$/MeOH/water (65 : 35 : 10, lower layer). The chromatograms of these compounds were quantitatively assayed with a TLC scanner after spraying with 5% $H_2SO_4$ solution followed by heating in a dry oven at 110 °C for 15 min.

**Extraction and Isolation of KPB and KPH from *Kalopanax pictus*** Dried stem bark (9.6 kg) of *K. pictus* was extracted three times with MeOH under reflux. The MeOH extract was filtrated and evaporated on a rotary evaporator under reduced pressure to obtain a viscous mass (1.28 kg). This residue was suspended in distilled water, 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 an *n*-BuOH-soluble fraction (450 g).

Part of the *n*-BuOH-soluble fraction (20 g) was fractionated by silica-gel (Merk Art 7734, Germany) column chromatography (7×60 cm) with the elution solvent, $CHCl_3$/MeOH/$H_2O$ (65 : 35 : 10, lower phase), to give subfraction 1 (0.23 g), subfraction 2 (5.26 g), subfraction 3 (1.93 g) and subfraction 4 (2.61 g). Subfractions 2 and 4 which gave a reddish color with the Lieberman–Burchard reaction were further purified by a combination of Sephadex LH-20 (3×42 cm) and ODS column chromatography (4×45 cm) and then recrystallized with MeOH to yield KPB (2.87 g, amorphous) and KPH (1.20 g, amorphous), respectively. By comparison of the physico-chemical data in the literature,[2,5] the two derivatives of KPB and KPH were identified as 2-*O*-[$\alpha$-L-rhamnopyranosyl-(1→2)-$\alpha$-L-arabinosyl]-hederagenin-28-*O*-[$\alpha$-L-rhamnopyranosyl-(1→4)-$\beta$-D-glucopyranosyl (1→6)-$\beta$-D-glucopyranoside and 3-*O*-$\beta$-[D-xylopyranosyl-(1→3)-$\alpha$-L-rhamnopyranosyl-(1→2)-$\alpha$-L-arabinosyl]-hederagenin-28-*O*-[-$\alpha$-L-rhamnopyranosyl-(1→4)-$\beta$-D-glucopyranosyl (1→6)-$\beta$-D-glucopyranoside, respectively. The latter compound was isolated for the first time from *K. pictus* by Porzel *et al.*[5] Part of the *n*-BuOH-soluble fraction (20 g) was fractionated by silica-gel (Merk Art 7734, Germany) column chromatography with the elution solvent $CHCl_3$/MeOH/$H_2O$ (65 : 35 : 10, lower phase).

KPB: Amorphous powder, mp 204—212 °C (dec.), $[\alpha]_D^{20}$ −18° (MeOH, $c$=0.69), IR $\upsilon_{max}^{KBr}$ $cm^{-1}$: 3416(broad, OH), 1728(COOH), 1054(glycoside); $^1$H-NMR(300 MHz, pyridine-$d_5$) $\delta$: see Table 1; $^{13}$C-NMR(75.5 MHz, pyridine-$d_5$) $\delta$: see Table 2.

KPH: Amorphous powder, mp 212—217 °C (dec.), $[\alpha]_D^{20}$ −25° (MeOH, $c$=0.96), IR $\upsilon_{max}^{KBr}$ $cm^{-1}$: 3417(broad, OH), 1729(COOH), 1052(glycoside); $^1$H-NMR(300 MHz, pyridine-$d_5$) $\delta$: see Table 1; $^{13}$C-NMR(75.5 MHz, pyridine-$d_5$) $\delta$: see Table 2.

**Extraction and Isolation of Metabolites** To obtain the metabolites of KPB or KPH produced by human intestinal bacteria, the reaction mixture contained 0.4 mg/ml KPB (or KPH) and 2 g fresh human fecal bacteria in a final volume of

Table 2. $^{13}$C-NMR Chemical Shifts of Kalopanaxsaponin B, H and Their Metabolites

| Position | HA | KPA | KPI | KPB | KPH |
|---|---|---|---|---|---|
| Aglycone | | | | | |
| C-1 | 38.7 | 38.9 | 38.9 | 38.9 | 38.9 |
| 2 | 26.0 | 26.1 | 26.1 | 26.0 | 25.9 |
| 3 | 81.7 | 81.0 | 81.0 | 81.0 | 81.6 |
| 4 | 43.4 | 43.4 | 43.5 | 43.3 | 43.4 |
| 5 | 47.5 | 47.7 | 47.6 | 47.5 | 47.5 |
| 6 | 18.0 | 18.1 | 18.0 | 18.0 | 18.3 |
| 7 | 32.8 | 32.8 | 32.8 | 32.4 | 32.4 |
| 8 | 39.7 | 39.7 | 39.7 | 39.8 | 39.8 |
| 9 | 48.1 | 48.1 | 48.1 | 48.0 | 48.1 |
| 10 | 36.8 | 36.8 | 36.8 | 36.7 | 36.8 |
| 11 | 23.7 | 23.7 | 23.7 | 23.6 | 23.7 |
| 12 | 122.5 | 122.5 | 122.5 | 122.7 | 122.7 |
| 13 | 144.7 | 144.8 | 144.7 | 144.0 | 144.0 |
| 14 | 42.0 | 42.1 | 42.0 | 42.0 | 42.0 |
| 15 | 28.2 | 28.3 | 28.2 | 28.1 | 28.2 |
| 16 | 23.8 | 23.8 | 23.7 | 23.2 | 23.2 |
| 17 | 46.6 | 46.6 | 46.5 | 46.1 | 46.1 |
| 18 | 41.8 | 41.9 | 41.9 | 41.5 | 41.5 |
| 19 | 46.5 | 46.6 | 46.5 | 46.1 | 46.1 |
| 20 | 30.8 | 30.9 | 30.9 | 30.6 | 30.6 |
| 21 | 34.1 | 34.2 | 34.1 | 33.9 | 33.9 |
| 22 | 33.1 | 33.2 | 33.2 | 32.6 | 32.6 |
| 23 | 64.3 | 63.9 | 63.9 | 63.9 | 63.9 |
| 24 | 13.5 | 13.9 | 14.1 | 13.7 | 14.0 |
| 25 | 16.0 | 16.0 | 16.0 | 16.0 | 16.1 |
| 26 | 17.4 | 17.4 | 17.4 | 17.4 | 17.4 |
| 27 | 26.0 | 26.1 | 26.1 | 25.9 | 25.9 |
| 28 | 180.1 | 180.3 | 180.1 | 176.5 | 176.5 |
| 29 | 33.1 | 33.2 | 33.2 | 33.0 | 33.0 |
| 30 | 23.8 | 23.6 | 23.7 | 23.7 | 23.7 |
| Sugar at C-3 of aglycone | | | | | |
| Ara-1 | 106.6 | 104.3 | 104.6 | 103.8 | 104.3 |
| 2 | 73.0 | 75.7 | 75.0 | 75.8 | 75.4 |
| 3 | 74.6 | 74.6 | 75.6 | 73.8 | 74.3 |
| 4 | 69.6 | 69.2 | 69.5 | 69.0 | 69.3 |
| 5 | 66.9 | 65.6 | 66.3 | 64.9 | 66.2 |
| Rha-1 | | 101.6 | 101.3 | 101.4 | 101.0 |
| 2 | | 72.3 | 71.9 | 72.0[a] | 71.9 |
| 3 | | 72.5 | 83.0 | 72.5[a] | 82.7 |
| 4 | | 74.0 | 72.9 | 73.8 | 72.5 |
| 5 | | 69.6 | 69.7 | 69.6 | 69.6 |
| 6 | | 18.5 | 18.4 | 18.3 | 18.2 |
| Xyl-1 | | | 107.6 | | 107.1 |
| 2 | | | 75.3 | | 75.2 |
| 3 | | | 78.4 | | 77.0 |
| 4 | | | 71.1 | | 70.7 |
| 5 | | | 67.4 | | 67.1 |
| Sugar at C-28 of aglycone | | | | | |
| Glc-1 | | | | 95.4 | 95.5 |
| 2 | | | | 75.0 | 75.0 |
| 3 | | | | 78.4 | 78.4 |
| 4 | | | | 70.1[b] | 70.5[b] |
| 5 | | | | 76.3 | 76.2 |
| 6 | | | | 70.6 | 71.0 |
| Glc-1 | | | | 104.4 | 104.5 |
| 2 | | | | 73.6 | 73.6 |
| 3 | | | | 77.8 | 77.8 |
| 4 | | | | 78.4 | 78.2 |
| 5 | | | | 76.8 | 76.9 |
| 6 | | | | 61.2 | 61.0 |
| Rha-1 | | | | 102.5 | 102.5 |
| 2 | | | | 72.2[a] | 72.1[a] |
| 3 | | | | 72.3[a] | 72.3[a] |
| 4 | | | | 73.7 | 73.5 |
| 5 | | | | 69.0[b] | 69.1[b] |
| 6 | | | | 18.3 | 18.2 |

*a*, *b*) Values may be interchanged in each column.

500 ml of anaerobic dilution medium. The mixture was incubated at 37 °C for 36 h and the reaction mixture was periodically extracted three times with ethylacetate.

The EtOAc-soluble portion of the KPB-reaction mixture was dried on a rotary evaporator under reduced pressure and subjected to silica-gel column chromatography (2.5×20 cm) with $CHCl_3$/MeOH/$H_2O$ (7 : 3 : 1, lower phase) to give three subfractions. From these subfractions, hederagenin (colorless needle, 24 mg), HA (colorless needle, 8 mg) and KPB (amorphous, 45 mg) were obtained by recrystalization of each subfraction with MeOH, respectively. By the same procedure, the KPH-reaction mixture yielded four compounds; hederagenin (35 mg), HA (6 mg), KPB (5 mg) and KPI (colorless needle from MeOH, 17 mg). The compounds identified by comparison of the physico-chemical data in the literature[2,6,7,8] (mp, $[\alpha]_D^{20}$, $^1$H-NMR and $^{13}$C-NMR) are as follows: hederagenin, HA (hederagenin 3-*O*-$\alpha$-L-arabinopyranoside), KPB (kalopanaxsaponin B), and KPI (hederagenin 3-*O*-$\beta$-[D-xylopyranosyl-(1→3)-$\alpha$-L-rhamnopyranosyl-(1→2)-$\alpha$-L-arabinoside). We named this compound kalopanaxsaponin I.

HA: Colorless needles from MeOH, mp 228—230 °C (dec.), $[\alpha]_D^{20}$ +48° (MeOH, $c$=1.16), IR $\upsilon_{max}^{KBr}$ cm$^{-1}$: 3413 (broad, OH), 1696(COOH), 1052(glycoside); $^1$H-NMR (300 MHz, pyridine-$d_5$) $\delta$: see Table 1; $^{13}$C-NMR(75.5 MHz, pyridine-$d_5$) $\delta$: see Table 2.

KPA: Colorless needles from MeOH, mp 265—268 °C (dec.), $[\alpha]_D^{20}$ +18° (MeOH, $c$=0.3), IR $\upsilon_{max}^{KBr}$ cm$^{-1}$: 3415 (broad, OH), 1698(COOH), 1058(glycoside); $^1$H-NMR (300 MHz, pyridine-$d_5$) $\delta$: see Table 1; $^{13}$C-NMR(75.5 MHz, pyridine-$d_5$) $\delta$: see Table 2.

KPI: Colorless needles from MeOH, mp 218—220 °C (dec.), $[\alpha]_D^{20}$ +11° (MeOH, $c$=0.28), IR $\upsilon_{max}^{KBr}$ cm$^{-1}$: 3417 (broad, OH), 1695(COOH), 1050(glycoside); $^1$H-NMR (300 MHz, pyridine-$d_5$) $\delta$: see Table 1; $^{13}$C-NMR(75.5 MHz, pyridine-$d_5$) $\delta$: see Table 2.

**Time-Courses of the Metabolism of KPB and KPH by Human Intestinal Bacteria** The metabolizing activity of KPB or KPH by human intestinal bacteria was measured as follows. The assay mixture contained 8 ml 0.4 mg/ml KPB (or KPH) and 0.1 g fresh human fecal bacteria in a final volume of 20 ml of an anaerobic dilution medium. The mixture was incubated at 37 °C for 36 h and an aliquot (1 ml) of the reaction mixture was periodically extracted twice with 5 ml ethylacetate. The ethylacetate fraction was analyzed by TLC.

**Screening of Intestinal Bacterial Strains Metabolizing KPB** According to our previous method,[9] a suspension of fresh feces from a Korean male was diluted $10^5$- to $10^8$-fold with GAM broth. An aliquot (200 $\mu l$) of $10^7$-diluted human feces was inoculated in a BL agar plate, which was anaerobically incubated at 37 °C for 4 d. Each colony was incubated in 5 ml GAM broth containing 0.4% KPB. The cultured medium was extracted with 5 ml ethylacetate. After evaporating the ethylacetate fraction, it was analyzed for KPB and its metabolites by TLC. Two bacteria, JY-6 and K-111, were isolated. Identification of the isolated bacterium was performed according to Bergey's manual.[10] JY-6 and K-111 were *Bacteroides spp.* and *Bifidobacterium spp.*, respectively (Table 3).

**Animals** Male Sprague-Dawley. (SD) rats (200—250 g) were supplied from Daehan Experimental Animal Breeding Center. All rats were housed in wire cages, fed with usual laboratory chow (Samyang Feed Production Co.) and water ad libitum.

**Induction and Treatment of Streptozotocin-Induced Diabetic Rats** Male SD rats were fasted overnight and injected intravenously with 50 mg streptozotocin per kg body weight. Streptozotocin was dissolved in citrate buffer (pH 4.5) and administered over 15 min. Control rats were treated with the same volume of citrate buffer. 72 h later, the blood glucose level of the treated rats was determined. Animals with a glucose level higher than 300 mg/dl were used as diabetic rats.

Rats were divided into seven groups and each group consisted of 8 experimental animals; normal control, diabetic control, diabetic KPH-treated (25 mg/kg), diabetic KPB-treated (50 mg/kg), diabetic kalopanaxsaponin A-treated (25 mg/kg), diabetic hederagenin-treated (50 mg/kg) and diabetic oleanic acid-treated (50 mg/kg). Samples were given intraperitoneally daily for 3 d to the diabetic rats and then they were killed 24 h after the final treatment with each sample.

**Determination of Glucose, Cholesterol, Lipoprotein–Cholesterols, Total Lipids and Triglycerides** Serum glucose levels were measured with a commercially available One Touch II (Lifescan) blood glucose strip test. Aliquots of serum were assayed enzymatically for total cholesterol,[11] lipoprotein–cholesterols,[12] total lipids[13] and triglycerides.[14] All experiments were conducted in duplicate.

## RESULTS AND DISCUSSION

**Metabolites of KPH and KPB Produced by Human Intestinal Bacteria** To investigate the metabolites of KPB produced by human intestinal bacteria, KPB was anaerobically incubated for 20 h with a bacterial mixture from human feces. Then the metabolites were extracted with ethylacetate and analyzed by TLC.

Three metabolites, two major and one minor, were observed on TLC. The *Rf*s of the major metabolites were 0.68 and 0.89 on TLC, respectively. $^1$H-NMR and $^{13}$C-NMR analysis identified them as KPA and hederagenin, respectively. The *Rf* of the minor metabolite was 0.80 on TLC and it was identified as HA by $^1$H-NMR and $^{13}$C-NMR analysis.

We also investigated the metabolites of KPH produced by human intestinal bacteria, KPH was anaerobically incubated for 20 h with a bacterial mixture from human feces. Then the metabolites were extracted with ethylacetate and analyzed by TLC.

Four metabolites, two major and two minor, were observed on TLC. The *Rf*s of the major metabolites were 0.60 and 0.89 on TLC, respectively. $^1$H-NMR and $^{13}$C-NMR analysis identified them as KPI and hederagenin, respectively. The *Rf*s of the minor metabolites were 0.68 and 0.80 on TLC, respectively. $^1$H-NMR and $^{13}$C-NMR analysis identified them as KPA and HA, respectively.

**The Time-Course of the Metabolism of KPB and KPH by Human Intestinal Bacteria** The time-course of the metabolism of KPB by human intestinal bacteria is shown in Fig. 1A. KPB began to be converted to KPA. The major metabolite was KPA and the minor metabolites were HA and hederagenin after a 6 h incubation with bacterial mixture. The main metabolites were KPA and hederagenin after a 12 h incubation. After 36 h, KPA and hederagenin were the main metabolites.

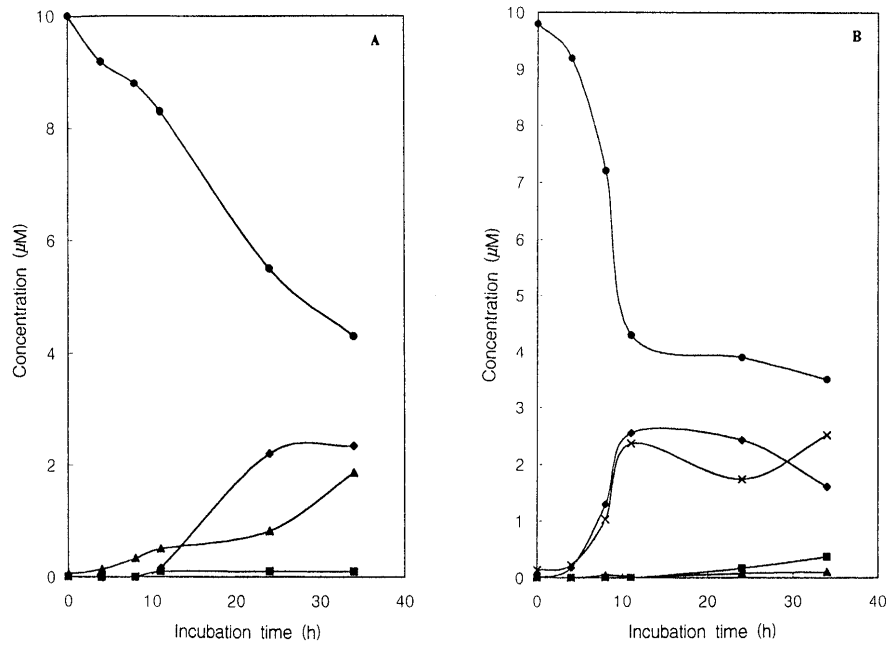


Fig. 1. Time-course of the Metabolism of KPB (A) and KPH (B) by Human Intestinal Bacteria

In (A), symbols are as follows: ●, KPB; ▲, KPA; ■, HA; ◆, hederagenin. In (B), symbols are as follows: ●, KPH; ×, KPI; ▲, KPA; ■, HA; ◆, hederagenin.

Table 3. Characteristics of *Bacteroides* JY-6 and *Bifidobacterium* K-111 Isolated from Human Intestinal Microflora

| Characteristic | JY-6 | K-111 |
|---|---|---|
| Shape | Rod | Rod |
| Oxygen | Anaerobe | Anaerobe |
| Gram stain | − | + |
| MR test | − | + |
| VP test | − | − |
| Nitrate reduction | + | − |
| Indole production | − | − |
| HS production | − | − |
| Simmons' citrate | − | − |
| β-Glucosidase | + | + |
| α-Rhamnosidase | + | + |
| Urease | − | − |
| Catalase | + | − |

The time-course of the transformation of KPH by human intestinal bacteria is shown in Fig. 1B. KPH began to be converted to KPI. The major metabolite was KPI and the minor metabolites were KPA, KPI and hederagenin after a 6 h incubation with the bacterial mixture. The main metabolites were KPI and hederagenin after 12 h incubation and, after 36 h, KPI and hederagenin were the main metabolites.

Then the intestinal bacteria that could transform KPB and KPH to hederagenin were isolated (Table 3). K-111 transformed KPB to hederagenin. Early on, KPB was metabolized to KPA and then to HA and hederagenin. The main metabolite was KPA after a 6 h incubation. After 36 h, KPA, HA and hederagenin were the minor metabolites.

JY-6 transformed KPB to hederagenin. Early on, KPB was metabolized to KPA *via* an unidentified metabolite and then gave HA and hederagenin. The main metabolites were KPA and the unidentified compound after a 6 h incubation. After 36 h, KPA and hederagenin were the main metabolites.

Using K-111, KPH was transformed to hederagenin. Early on, KPH was metabolized to KPI and then to KPA, HA and hederagenin. The main metabolite was KPI after a 6 h incubation. After 36 h, the main metabolites were KPI and hederagenin.

JY-6 transformed KPH to hederagenin. Early on, KPH was metabolized to KPI *via* an unidentified metabolite and then to KPA, HA and hederagenin. The main metabolites were KPI and the unidentified compound after a 6 h incubation. After 36 h, the main metabolites were KPI and hederagenin.

**Metabolic Pathway of KPB and KPH** When KPB was incubated with human intestinal bacteria, three metabolites were produced. The time-course of KPB metabolism by human intestinal bacteria was as follows: Early on, KPB was converted to KPA, and then to HA and hederagenin.

When KPH was incubated with human intestinal bacteria, four metabolites were produced. The time-course of KPH metabolism by human intestinal bacteria was as follows: Early on, KPH was converted to KPI, and then to KPA, HA and hederagenin.

From these results, the proposed metabolic pathway of KPB and KPH by human intestinal bacteria is shown in Chart 1.

Using intestinal bacteria, KPB was mainly transformed to KPA and hederagenin. KPH was mainly transformed to KPI and hederagenin. If a water extract of *K. pictus*, containing KPB and KPH, was administered, KPB should be metabolized to KPA and hederagenin, and KPH to KPI and hederagenin, respectively.

**Antidiabetic Activity of the Metabolites of Kalopanaxsaponin B and H Produced by Human Intestinal Bacteria** To investigate the role of intestinal bacteria in the biological activity of kalopanaxsaponin B and H, the antidiabetic activity of these compounds and their metabolites transformed by human intestinal bacteria was measured in the streptozotocin-induced diabetic rat (Table 4). These compounds were administered intraperitoneally. The antidiabetic activity of KPA was highest, followed by hederagenin. However, KPB and KPH had no activity. First of all, as far as hypoglycemic

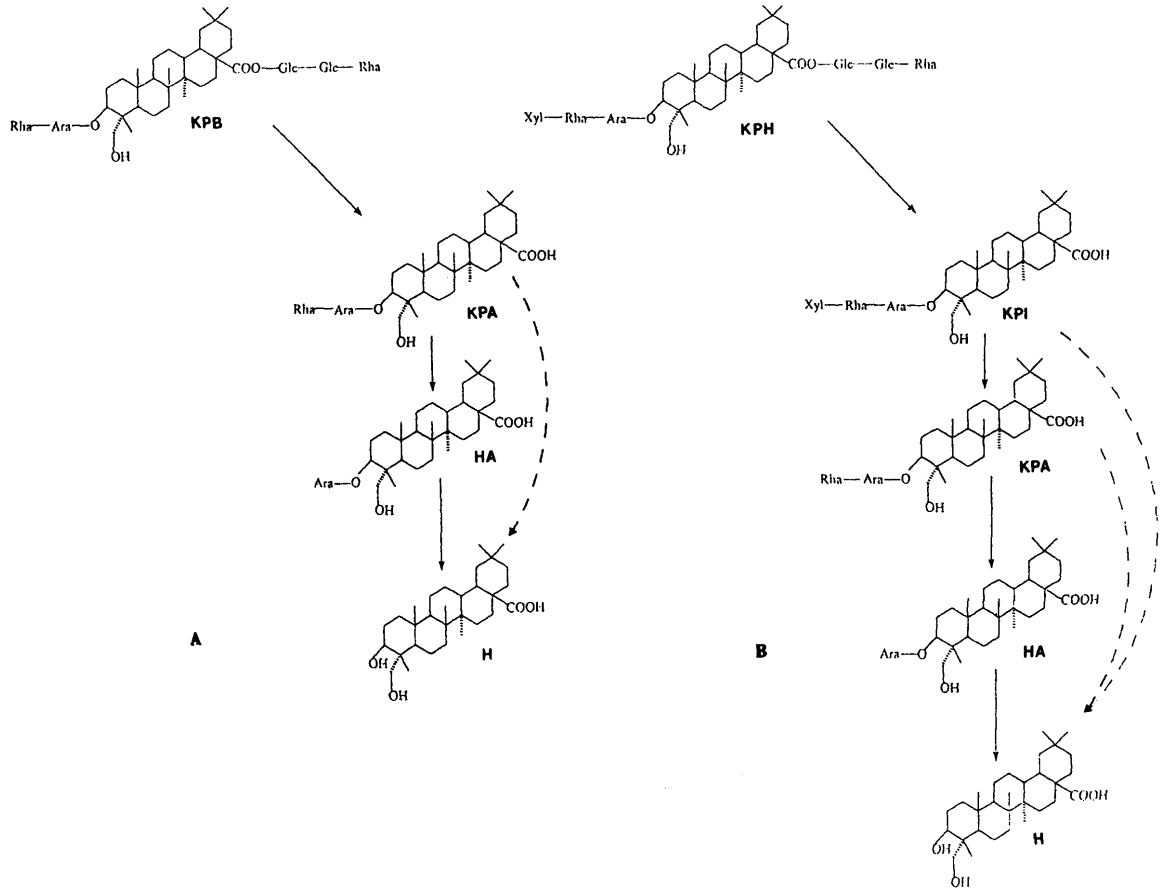


Chart 1. Possible Metabolic Pathways of KPB (A) and KPH (B) Produced by Human Intestinal Microflora
Main pathway, —; minor pathway, ---; H, hederagenin.

Table 4. 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±10.59[a)] |
| Streptozotocin | | 323.3±52.52[b)] |
| Oleanic acid | 50 | 316.6±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)] |

*a*) Values are means±S.D. ($n$=8). *a,b,c,d*) Those with the same letter are not significantly different at $p<0.05$.

Table 6. Effect of the Constituents of the Stem Bark of *Kalopanax pictus* on the 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±26.46[a)] | 74.7±12.88[a)] |
| Streptozotocin | | 341.4±44.29[b,c)] | 132.2±20.33[b)] |
| Oleanic acid | 50 | 320.9±28.21[b,c,d)] | 121.0±14.62[b,c)] |
| Hederagenin | 50 | 304.6±18.31[b,c)] | 107.1±10.84[c,d)] |
| Kalopanaxsaponin A | 25 | 289.2±13.39[a,d)] | 99.0±10.03[d)] |
| Kalopanaxsaponin B | 50 | 317.9±17.85[b,c,d)] | 128.1±17.38[b,c)] |
| Kalopanaxsaponin H | 25 | 339.6±38.74[b,c)] | 119.8±13.25[b,c,d)] |

*a*) Values are means±S.D. ($n$=8). *a,b,c,d*) Those with the same letter are not significantly different at $p<0.05$.

Table 5. Effect of the Constituents of the Stem Bark of *Kalopanax pictus* on the 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)] |
| Oleanic 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)] |

*a*) Values are means±S.D. ($n$=8). *a,b,c,d*) Those with the same letter are not significantly different at $p<0.05$.

activity is concerned, KPA significantly reduced the serum glucose level which markedly elevated by streptozotocin administration. The serum glucose level of this group was similar to that of the normal group.

In addition, KPA effectively reduced the level of total serum cholesterol and (VLDL+LDL) cholesterol in the streptozotocin-induced diabetic rat (Table 5). However, HDL-cholesterol was increased 49%. KPA reduced the total lipids and triglycerides level by 58% and 57%, respectively (Table 6). Hederagenin also reduced the glucose level, total cholesterol, (VLDL+LDL) cholesterol, total lipids and triglycerides. However, the antidiabetic activity of hederagenin was weaker than that of KPA. These results suggest that KPA and hederagenin could improve the hypercholesterolemia and hyperlipidemia due to diabetes mellitus.

In Korea, the stem bark of *K. pictus*, which contains many hederagenin glycosides, such as KPB and KPH, has been used as an antidiabetic agents. However, when these constituents, KPB and KPH, were administered *i.p.*, there was no antidiabetic activity. Furthermore, on orally administering a water extract of the stem bark of *K. pictus*, KPB and KPH were metabolized to KPA and hederagenin. This biotransformation occurs in the human intestine due to intestinal bacteria and so these metabolites could play an important role as antidiabetic agents. Finally, we are convinced that KPB and KPH in *K. pictus* are natural prodrugs for the antidiabetic effect.

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