# Saponins from Leaves of *Kalopanax pictum* var. *maximowiczii*, a Korean Medicinal Plant

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**From leaves of *Kalopanax pictum* var. *maximowiczii*, a Korean medicinal plant, six known saponins of hederagenin were isolated. One of the monodesmosides was identified as sapindoside A, previously isolated from *Sapindus* spp. Another monodesmoside and four bisdesmosides were proved to be identical with saponins-$K_3$, -$K_{10}$ and -$K_{12}$ and Kizuta saponins-$K_8$ and -$K_{11}$, respectively, all of which have been isolated from *Hedera rhombea*. It was observed that the water solubilities of these monodesmosides were increased in the presence of the co-occurring bisdesmosides. The relationship between structure and solubilizing effect is reported.**

**Keywords** *Kalopanax pictum* var. *maximowiczii*; Araliaceae; Korean medicinal plant; saponin; hederagenin; sapindoside A; kizuta saponin; solubilizing effect

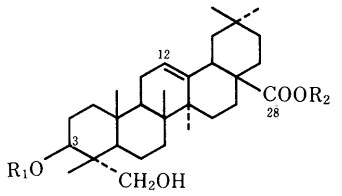
*Kalopanax pictum* NAKAI var. *maximowiczii* NAKAI (Korean name: hae dong), grows in western Korea and its stem bark has been used as a folk medicine. As a part of our serial studies on the chemical constituents of oriental araliaceous plants,[1,2] the present paper describes the isolation of triterpene saponins from leaves of this plant. Solubilizing properties of the saponins are also reported.

The leaves, collected in the suburbs of Seoul, were extracted with methanol and a suspension of the methanolic extract in water was extracted with ethyl acetate and then with 1-butanol saturated with water. The ethyl acetate fraction was washed with hexane and chromatographed on silica gel and then on reverse-phase silica gel to give two saponins, **1** and **2**. Based on a comparison of the $^1$H- and $^{13}$C-nuclear magnetic resonance ($^1$H- and $^{13}$C-NMR) spectra with those of an authentic sample, **1** was identified as sapindoside A, a monodesmosidic saponin of hederagenin (**3**) from pericarps of *Sapindus* spp. (Sapindaceae).[3] Inspection of the $^1$H- and $^{13}$C-NMR spectra led to the identification of **2** as saponin-$K_3$ (3-*O*-α-arabinopyranoside of **3**) previously isolated from *Hedera rhombea* BEAN (Araliaceae).[4]

The butanol fraction was chromatographed on silica gel to give four saponins, **4**—**7**. Based on the $^1$H- and $^{13}$C-NMR spectra, these saponins **4**—**7** were identified as saponin-$K_{10}$,[4] kizuta saponins-$K_8$ and -$K_{11}$[5] and saponin-$K_{12}$,[4] respectively, all of which have already been isolated from *Hedera rhombea*. Saponins **4** and **7** are bisdesmosides which correspond to **2** and **1**, respectively. Saponins **5** and **6** are formulated as monoacetylated **4** and **7**, respectively.

Previously, from pericarps of *Sapindus mukurossi* GAERTN, we have isolated monodesmosides[3] of **3** which are sparingly soluble in water. It was found that the water solubilities of these monodesmosides were greatly increased in the presence of the corresponding bisdesmosides which co-occur in the pericarps.[3,6] In the present study, the solubilizing effect of the bisdesmosides **4**—**7** on the monodesmosides **1** and **2** was investigated and the results are summarized in Tables I and II.

Water solubility of **1** was greatly increased in the presence of the corresponding bisdesmoside, **7**. This solubilizing effect was significantly decreased by monoacetylation of the sugar moiety (to **6**) or by loss of a terminal rhamnosyl unit (to **4**) and the solubilizing effect was hardly observed with the desrhamno-monoacetyl saponin, **5**. The bisdes-




| | $R_1$ | $R_2$ |
|---|---|---|
| **1** | α-L-Ara(2→α)-L-Rha | H |
| **2** | α-L-Ara | H |
| **3** | H | H |
| **4** | α-L-Ara | β-D-Glc(6→β)-D-Glc(4→α)-L-Rha |
| **5** | α-L-Ara | β-D-Glc(6→β)-D-Glc(4→α)-L-Rha, 6Ac |
| **6** | α-L-Ara(2→α)-L-Rha | β-D-Glc(6→β)-D-Glc(4→α)-L-Rha, 6Ac |
| **7** | α-L-Ara(2→α)-L-Rha | β-D-Glc(6→β)-D-Glc(4→α)-L-Rha |

Chart 1

TABLE I. Solubilizing Effect of Bisdesmosides (**4**—**7**) of *Kalopanax pictum* var. *maximowiczii* on Sapindoside A (**1**) (in Water at 37 °C)

| Bisdesmoside | Conc. of bisdesmoside mg/ml [mM] | Solubility of sapindoside A μg/ml [mM] |
|---|---|---|
| None | — | 31.2 [0.04] |
| **5** | 0.5 [0.45] | 43.9 [0.06] |
| | 1.0 [0.89] | 50.5 [0.07] |
| **4** | 1.0 [0.93] | 238.8 [0.32] |
| **6** | 0.5 [0.40] | 197.5 [0.26] |
| | 1.0 [0.79] | 317.7 [0.42] |
| **7**[a] | 0.5 [0.41] | 1287.5 [1.71] |
| | 1.0 [0.82] | 3920.0 [5.22] |

*a*) The amount of **1** added was 1.0 mg/ml except for the case of **7** (5.0 mg/ml).

TABLE II. Solubilizing Effect of Bisdesmosides (**4**—**7**) of *Kalopanax pictum* var. *maximowiczii* on Saponin-$K_3$ (**2**) (in Water at 37 °C)

| Bisdesmoside | Conc. of bisdesmoside mg/ml [mM] | Solubility of saponin-$K_3$ μg/ml [mM] |
|---|---|---|
| None | — | 2.1 [0.003] |
| **5** | 1.0 [0.89] | 116.2 [0.19] |
| **4** | 1.0 [0.93] | 123.5 [0.20] |
| **6** | 1.0 [0.79] | 125.9 [0.21] |
| **7** | 1.0 [0.82] | 132.5 [0.22] |

The amount of **2** added was 1.0 mg/ml.

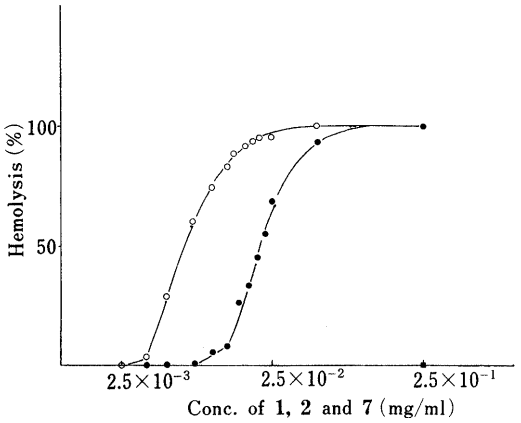



Fig. 1. Hemolysis by Saponins **1**, **2** and **7**

Sheep erythrocytes were incubated in isotonic phosphate buffer (pH 7.4) at 37 °C for 30 min. Saponins **1** and **2** were solubilized with the aid of **7** —○—, **1**; —●—, **2**; —■—, **7**.

mosides, **4**—**7** also solubilized the monoarabinoside **2**, which is almost insoluble in water. However, in contrast to the case of **1**, no significant difference in the solubilizing effect was observed between these bisdesmosides. No solubilizing effect on Yellow OB or saikosaponin a was observed with these bisdesmosides.

Hemolytic activities of the monodesmosides **1** and **2** solubilized with the aid of **7**, which exhibits no hemolysis, are shown in Fig. 1.

## Experimental

**Isolation of Saponins** The dried leaves (3 kg) collected in the suburbs of Seoul, Korea, were extracted with hot MeOH. A suspension of the MeOH extract in water was extracted with EtOAc and then with 1-BuOH saturated with water.

The EtOAc fraction was washed with $C_6H_{14}$ and the insoluble substances (4.85 g) were subjected to chromatography on silica gel with AcOEt–EtOH–$H_2O$ (80 : 10 : 1, homogeneous) and then on LiChroprep RP-8 (40—63 $\mu$m, Merck) with 60% MeOH to give **1** and **2** in yields of 0.018 and 0.003%, respectively.

**1**: Colorless needles from 70% MeOH, mp 248—249 °C, $[\alpha]_D^{20}$ +18.5° ($c$=0.65, MeOH). **2**: Colorless needles from 70% MeOH, mp 232—233 °C, $[\alpha]_D^{21}$ +82.0° ($c$=0.90, $C_5H_5N$).

The BuOH fraction (5.95 g) was chromatographed twice on silica gel; first with $CHCl_3$–MeOH–$H_2O$ (20 : 10 : 1, homogeneous) and then with $CHCl_3$–MeOH–$H_2O$ (65 : 30 : 1, lower phase) to give **4**, **5**, **6** and **7** in yields of 0.004, 0.04, 0.06 and 0.044%, respectively.

**4**: A white powder, $[\alpha]_D^{21}$ +10.1° ($c$=1.02, $C_5H_5N$). **5**: A white powder, $[\alpha]_D^{20}$ −2.1° ($c$=2.46, MeOH). **6**: A white powder, $[\alpha]_D^{20}$ −22.0° ($c$=1.40, MeOH). **7**: A white powder, $[\alpha]_D^{20}$ −8.2° ($c$=1.51, $C_5H_5N$).

Identification of each of the saponins were established by comparison of the $^1$H- and $^{13}$C-NMR spectra (in $C_5D_5N$) and optical rotation with reference data.

**Solubilization of Monodesmosides (1 and 2) with Bisdesmosides (4—7)** Saturated aqueous solutions of **1** and **2** were prepared by incubation of an excess of each saponin in $H_2O$ (5 ml) at 37 °C for 24 h followed by filtration to remove undissolved saponin. Saturated solutions of the monodesmosides in an aqueous solution of a bisdesmoside were prepared as follows. A solution of an excess of a monodesmoside in MeOH containing a bisdesmoside was concentrated to dryness and the residue was incubated in $H_2O$ (5 ml) at 37 °C for 24 h. Each solution was filtered to give a saturated solution.

The concentration of the monodesmoside in each saturated solution was determined by thin layer chromatography (TLC)-densitometry under the following conditions (the procedure is similar to that used for analysis of Ginseng saponins[7] and Sapindus saponins[3,6]). A MeOH solution of each monodesmoside was spotted on a silica gel TLC plate (20 × 20 cm, $60F_{254}$ precoated, Art.11798 Merck) and developed with $CHCl_3$–MeOH–$H_2O$ (30 : 10 : 1, homogeneous). After being sprayed with 10% $H_2SO_4$, the plate was heated at 110 °C for 10 min, immediately covered with a glass plate of the same size to prevent color change of the spot and then subjected to TLC-densitometry with a Shimadzu CS-930 dual-wavelength TLC scanner (reflection mode: $\lambda$s, 530 nm; $\lambda$r, 700 nm. Calibration plots of integrated values of spots against weight/spot were found to be linear for **1** and **2** up to a concentration of 1 $\mu$g/spot, and the extrapolated plots passed through the origin.

**Solubilizing Effect on Yellow OB and Saikosaponin a** See previous papers.[6,8]

**Determination of Hemolysis of Commercial Sheep Erythrocytes** This was done in phosphate buffer (pH 7.4) at 37 °C, see a previous paper.[9]

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