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Saponins from the Stem Bark of *Kalopanax pictum* var. *magnificum* (I)

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Abstract □ Three triterpenoid saponins were isolated from the methanol extract of the stem bark of *Kalopanax pictum* Nakai var. *magnificum* (Araliaceae). The structures of these saponins were identified as hederagenin 3-O-α-L-arabinopyranoside, hederagenin-3-O-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside and 3-O-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl hederagenin 28-O-α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl ester.

Keywords □ *Kalopanax pictum* Nakai var. *magnificum*, Araliaceae, hederagenin monodesmosides, hederagenin bisdesmoside, Korean folk medicine, Kalopanacis Cortex.

Kalopanax pictum Nakai var. *magnificum* is a deciduous tree of the family Araliaceae, which is distributed in the middle zone of Korean peninsula. The stem bark of this plant has been used in traditional Korean medicine for neuralgia, lumbago, arthritis, tonic and antidiabetes under the name of Kalopanacis Cortex[1].

Several saponins have been isolated in the species[2-6,9-11]. In the provious paper we reported the isolation and identification of one hederagenin monodesmoside (α-hederin), hederagenin bisdesmoside (pentaglycoside), and (+)-syringaresinol di-β-D-glucoside from *Kalopanax pictum* var. *maximowiczii*[7,8].

This paper deals with the isolation of two monodesmosides and one bisdesmoside from the title plant, which has been identified on the basis of chemical and spectral evidences.

The TLC chromatogram of methanol extract from the stem bark of the title plant indicated the presence of eight kinds of saponins, which were tentatively named saponins A-H. Among them three kinds of saponins A (**1**) mp 230-233℃, B (**2**) mp 248-252℃, and D (**3**) mp 226-228℃, were isolated in the pure state by the fractional procedure on the Amberlite XAD-2 column followed by repeated SiO_2 column chromatography. Acid hydrolysis of these saponins gave the same aglycone, hederagenin (**4**), identified by comparison with an authentic sample, and from the glycone part, arabinose from **1**, arabinose and rhamnose from **2**, and arabinose, rhamnose and glucose (1:2:2) from **3** were identified by TLC.

The ^{1}H-NMR spectra of **1** and **2** showed six tertiary methyl groups at δ 0.88-1.17 (CH_3×6) and one anomeric proton signal at δ 4.99 from **1**, and two anomeric proton signals at δ 5.09 and 6.15 from **2**. The ^{1}H-NMR spectrum of **3** showed the same pattern as that of **2** with additional another three anomeric proton signals. These results suggested that **1**, **2** and **3** were mono-, di- and pentaglycoside with the same aglycone.

The ^{13}C-NMR signals of **1**, **2** and **3** revealed that **1** and **2** are monodesmoside, and **3** is bisdesmoside. The δ values of C-28 of **1**, **2** and **3** were 180.4, 180.4 and 176.5, respectively. The formation of the ester linkage at C-28 in **3** resulted in the chemical shift down verified that suggested **3** is a bisdesmoside. The anomeric carbon signals due to the glycone moiety of these compounds indicated the presence of one and two monosaccharide units in **1**, **2** and five monosaccharide units in **3**.

Methanolysis of these saponins permethylate **5** (from **1**), **6** (from **2**), **7** (from **3**) gave the same aglycone methyl ester, 23-*O*-methyl hederagenin methyl ester (**8**) and methyl 2,3,4-tri-*O*-methyl-L-arabinoside from **5**, methyl pyranosides of 2,3,4-tri-*O*-methyl rhamnose and 3,4-di-*O*-methyl arabinose from **6** and methyl pyranosides of 2,3,4-tri-*O*-methyl rham-

nose, 3,4-di-*O*-methyl-L-arabinose, 2,3,6-tri-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-D-glucose from 7, respectively.

The ^{1}H-NMR spectrum of **5** showed the anomeric proton signal at δ 4.99 (1H, d, J=7.0 Hz) whose coupling constant indicates that arabinose in **1** is α-linked to aglycone. Consequently, saponin **1** is hederagenin 3-*O*-α-L-arabinopyranoside. The methylation analysis showed that saponin **2** contained a terminal rhamnose group and 2-linked arabinopyranosyl residue. Furthermore, the ^{13}C-NMR spectra of **2** with the terminal glycosyl group of authentic methylglycoside revealed that the terminal sugar is α-rhamnose. Therefore, the structure of 2 is 3-*O*-α-L-rhamnosyl(1→2)-α-L-arabinosyl-23-hydroxyolean-12-en-28-oic acid, identified by a direct comparison of an authentic sample obtained from *Kalopanax pictum* and *Hedera rhombea*[12].

On the alkaline hydrolysis of **3** affored **2** and trisaccharide (anhydrous form). Acid hydrolysis of trisaccharide afforded rhamnose and glucose. Selective cleavage[13] of **3** (ester type glycosyl linkage) with anhydrous LiI and 2,6-lutidine in MeOH (anhyd.), gave **2** and methylglycoside (**3b**). Permethylation of **3b** gave a permethylate (**3c**). Acid hydrolysis of (**3c**) then reduction by $NaBH_4$ and acetylation by Ac_2O afforded a mixture of *O*-methyl alditol acetates. The GC-MS analysis of the mixture revealed the formation of *O*-methylalditol acetate to terminal rhamnopyranose, 4-linked glucopyranose and 6-linked glucopyranose (Fig. 1).

The mode of linkage of esterglycosyl glucose unit was regarded as β-form on the basis of the ^{1}H-NMR spectrum of **3** showing an anomeric proton signal of esterglycosyl glucose at δ 6.20 as a doublet (J=8.4 Hz)[14].

The ^{13}C-NMR-moiety is definitely different between α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl unit and α-L-rhamnopyranosyl(1→6)-β-D-glucopyranosyl(1→4)-β-D-glucopyranosyl unit[15,16]. On the basis of these results saponin **3** is assigned as 28-α-L-rhamnopyranosyl (1-4)-β-D-glucopyranosyl(1→6)-β-D-glusopyranoside of **2** and identified as 3-*O*-α-L-rhamnopyranosyl (1-2)-α-L-arabinosyl-23-hydroxyolean-12-en-*O*-α-L-rhamnopyranosyl(1→4)-*O*-β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl ester by direct comparison of the authentic sample, which was isolated from *Kalopanax pictum* var. *maximowiczii*.

Saponin **1** was isolated from *Kalopanax pictum*[2] and *Hedera rhombea* in the earlier reports[12]. Saponin **2** was isolated from the *Kalopanax pictum* var. *typicum*[3], *Akebia quinate*[17], *Hedera* plants[18-20], *Patrinia* sp.[21] *Pulsatilla koreana*[22], *Kalopanax pictum* var. *maximowiczii*[7], *Caltha palustris*[23]. Saponin **3** was also isolated from *Pulsatilla koreana*[22], *Kalopanax pictum* var. *maximowiczii*[6,7], *Pulsatilla cernua*[24].

EXPERIMENTAL

All melting points were measured with *FP5* (Mettler Co.) and are uncorrected. IR spectra were taken from FT infrared spectrometer Model Nicolet MZ-S. NMR spectra were recorded on a Varian XL 200 NMR spectrometer 200 MHz in pyridine-d_5, $CDCl_3$ solution and chemical shift are given in δ (ppm), with tetramethylsilan as an internal standard. Mass spectra was taken by JMS-D 3000 JEOL mass spectrometer. Gas liquid chromatogram was run on a Varian 1800 by SE-30, 80% chromosorb-W stainless column (5′×8″), Column temp. was 80-235℃ with FID detector. Carrier gas was He (45 ml/min). Elemental analysis was performed by Perkin Elmer 240EA and optical rotation was measured with a Rudolph Autopol-TM-III automatic polarimeter.

Extraction and isolation

The stem barks of *Kalopanax pictum* Nakai var.

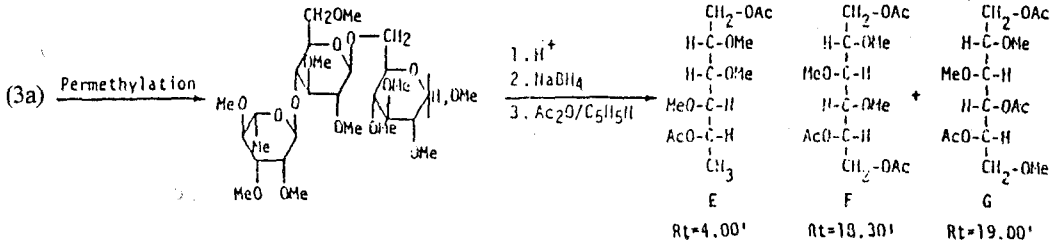

Fig. 1. Sugar sequence analysis by GC-MS.

magnificum collected in the Kwang-Nung forest in June, 1987 were extracted with hot MeOH. The extract was chromatographed on highly porus polymer (Amberlite XAD-2) using H_2O, 50% MeOH, 70% MeOH and 100% MeOH as elution solvents. Saponin **3** was eluated from 50% MeOH, **1** and **2** were from 70% MeOH, respectively. The saponins **1**, **2** and **3** were separated in a pure form by repeated column chromatography on SiO_2 gel (solvent, $CHCl_3$: MeOH:H_2O=70:30:4 or 60:40:10 by vol.). Total yields of 1, 2 and 3 were 0.10, 0.30, 0.25%, respectively.

Saponin A (1)

Colorless needles (MeOH), mp. 230-233℃, $[\alpha]_D$ +66.5° (*c* 0.30, MeOH). Anal. Calcd. for $C_{35}H_{56}O_8$ 1.5 H_2O, C 66.50: H 9.40, Found: C 66.23; H 9.30. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1692 (COOH). ^{1}H-NMR (pyridine-d_5): 0.69 (3H, s), 0.70 (3H, s), 0.80 (3H, s), 1.15 (6H, s), 1.24 (3H, s), 4.99 (1H, d, J=7.0 Hz, anomeric H), ^{13}C-NMR: see Table I.

Saponin B (2)

Colorless needles (from MeOH), mp. 248-252℃, $[\alpha]_D$+18.0° (*c* 0.10, pyridine). Anal. Calcd. for $C_{41}H_{66}O_{12}$: C 62.57: H 8.97. Found: C 62.50; H 9.40; IR ν_{max}^{KBr} cm^{-1}, 3400 (OH), 1700 (COOH), 1640 (C=C), 1100, 1027 (C-O). ^{1}H-NMR (pyridine-d_5): 0.69 (3H, s), 0.70 (3H, s), 0.80 (3H, s), 1.15 (6H, s), 1.25 (3H, s), 1.63 (3H, d, J=6.2 Hz, Me of rhamnose), 6.27 (1H, s, anomeric H), 5.16 (1H, d, J=7.0 Hz, anomeric H), ^{13}C-NMR: see Table I.

Saponin D (3)

A white powder, precipitated with EtOAc in MeOH solution. mp 226-228℃ $[\alpha]_D$−8.0° (pyridine). Anal. Calcd. for $C_{59}H_{96}O_{26}3H_2O$: C 55.56; H 8.25. Found: C 55.20; H 8.25; IR ν_{max}^{KBr} cm^{-1}, 3400 (OH), 1751 (ester), 1100 (C-O), 1645, 807 (C=C). ^{1}H-NMR (pyridine-d_5): 0.72 (3H, s), 0.98 (6H, s), 1.00 (3H, s), 1.02 (3H, s), 1.25 (3H, s), 1.65, 1.71 (each 3H, d, J=6.2 Hz, Me of rhamnose X 2), 4.96 (1H, d, J=7.7 Hz, anomeric H), 5.16 (1H, d, J=7.0 Hz, anomeric H), 5.81 (1H, br.s, anomeric H), 6.27 (1H, br.s, anomeric H), 6.20 (1H, d, J=7.8 Hz, anomeric H). ^{13}C-NMR: see Table I.

Acid hydrolysis of saponins 1, 2 and 3

Each saponin was hydrylized in a sealed tube with 2 N-HCl:dioxane (1:1), 90℃ for 4 h. Hederagenin (**4**) was identified as the genin of these saponin with chemicophysical data and direct comparison with the authentic sample. The mother liquid of hydrolysate was neutralized and evaporated, and then glycones of these saponins were identified as arabinose from **1**, arabinose and rhamnose from **2**, and arbinose, rhamnose and glucose from **3**.

Table I. ^{13}C-NMR chemical shift in pyridine-d_5

Carbon No.		Hederagenin	1	2	3
3		73.7	82.0	81.1	81.2
12		122.7	123.1	123.0	123.0
13		145.0	144.3	144.3	144.2
23		68.2	64.6	64.5	64.5
28		180.4	180.4	180.4	176.5
3-*O*-sugar moiety					
α-L-ara	1		106.8	104.3	104.2
	2		73.2[a]	75.8	75.8
	3		74.8[a]	74.8	74.5
	4		69.8	69.6	69.2
	5		66.8	65.6	65.4
α-L-ara	1			101.6	101.6
	2			72.2[a]	72.3[a]
	3			72.4[a]	72.5[a]
	4			74.0	74.1
	5			69.6	69.7
	6			18.5	18.4
28-*O*-sugar moiety					
β-D-glu					
(inner)	1				95.2
	2				73.8[a]
	3				78.7[a]
	4				70.9
	5				78.5
	6				69.5
β-*D*-glu					
(inter)	1				104.8
	2				75.4
	3				76.5
	4				78.3
	5				77.1
	6				61.3
α-*L*-rha					
(terminal)	1				102.4
	2				72.7
	3				72.8
	4				73.9
	5				70.2
	6				18.4

[a]Assignment may be reversed in each column.

Permethylation and methanolysis of saponins 1, 2 and 3

According to the Hokomori method[25], each sample 100 mg, DMSO 2.5 m*l* and NaH 150 mg streaming under N_2 was treated for 2 h in an ultrasonicator. After cooling in ice water, added 10 m*l* of CH_3I and allowed for another 1 h in the ultrasonicator. The reaction mixture was diluted with water and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, and dehydrated (Na_2SO_4). Dehydrated $CHCl_3$ layer was evaporated and recrystallized from MeOH to give their permethylates **5**, **6** and **7** as colorless needles. **5**: mp. 182℃; IR no OH, **6**: mp. 183-185℃; IR no OH, **7**: mp. 120-122℃; IR no OH. Methanolysis was performed separately. **5**, **6** and **7** (each 50 mg) was boiled with 8% HCl-MeOH (15 m*l*) in the water bath for 2 h. The hydrolysates were neutralized and evaporated. Each residue was recrystallized from MeOH to give colorless needles, mp. 186℃, 23-*O*-methylhederagenin methyl ester (**8**), **8** was identified as the methylated genin by a direct comparison with an authentic sample.

The methylated sugar in the mother liquid was identified as methyl 2,3,4-tri-*O*-methyl-L-arabinoside from **5**, methyl pyranoside of 2,3,4-tri-*O*-methyl-L-rhamnose, 3,4-di-*O*-methyl-*L*-arabinose from **6**, methyl pyranoside of 2,3,4-tri-*O*-methyl rhamnose, 3,4-di-*O*-methyl-L-arabinose, 2,3,6-tri-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-D-glucose from **7**.

Alkaline hydrolysis of 3

Alkaline hydrolysis of **3** (100 mg) was carried out by refluxing with 0.5 N-KOH in MeOH for 1 h. and diluted with water, neutralized (Amberlite MB-3), extracted with AcOEt:EtOAc *n*-BuOH (1:1), washed and concentrated to give hydroyized product, which was identified as **2**. The mother liquid was hydrolyzed with HCl, neutralized and rhamnose and glucose were characterized by TLC.

Selective cleavage of the ester glycoside linkage of 3

Saponin **3** (200 mg), anhydrous LiI (200 mg), 2,6-lutidine (18 m*l*) and MeOH (4 m*l*, anhydrous) was refluxed for 16 h. The resulted solution was deionized (Amberlite MB-3), concentrated to dryness, suspended in H_2O, chromatographed (Amberlite XAD-2) and eluted with H_2O gave methylglycoside (**3a**) and subsequent elution with MeOH afforded **2**. **3a** was permethylated as above mentioned.

3a permetylate was treated with 90% HCOOH (1.8 m*l*) at 100℃ for 1 h. The reaction mixture was evaporated to remove HCOOH under the reduced pressure. The residue was treated with 0.13 M-H_2SO_4 at 100℃ for 15 h, neutralized, washed with water. The filtrate and washing were combined, concentrated to about 3.7 m*l* and reduced with $NaBH_4$ (45 mg). It was placed at the room temperature for 2 h, acidified (Dowex 50 H^+ form), concentrated to dryness. Boric acid in the resudie was removed by treated codistillation with MeOH. The resulting methylated alditols mixture was acetylated with Ac_2 O-pyridine (1:1) at 100℃, for 1 h. The reagents were removed by codistillation with toluene. The methylated alditiol acetates mixture was subjected to GC-MS analysis. GC-MS was taken on Shimazu GC-MS 7000s, glass column 2.6×1.5 m packed with 5% ECNSS-M on Chromosorb W. injection temp. 200 ℃, column temp. 170℃, carrier gas He at 35 ml/min, separator temp. 250℃. Ionization voltage 70 eV. accelerating voltage 1.5 kV. The t_R of three methylated alditol acetate were 4.00 (from terminal rhamnose), 18.30 (from inner glucose), 19.00 (from inter glucose), respectively.

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