Saponins from Leaves of *Kalopanax pictus* (THUNB.) NAKAI, Harigiri: Structures of Kalopanax-Saponins JLa and JLb

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From leaves of *Kalopanax pictus* (THUNB.) NAKAI., collected in Japan, two new saponins named kalopanax-saponins JLa (4) and JLb (5) were isolated together with three known saponins, kalopanax-saponins A (1) and B (2) and kizuta saponin K_{11} (3). On the basis of chemical and spectral data, the structures of these new saponins were concluded to be as follows: 4: 3-*O*-α-arabinopyranosyl-(1→2)-α-rhamnopyranosyl hederagenin 28-*O*-(2-*O*-acetyl-α-rhamnopyranosyl)-(1→4)-(6-*O*-acetyl-β-glucopyranosyl)-(1→6)-β-glucopyranosyl ester; 5: 3-*O*-α-arabinopyranosyl-(1→2)-α-rhamnopyranosyl hederagenin 28-*O*-(3-*O*-acetyl-α-rhamnopyranosyl)-(1→4)-(6-*O*-acetyl-β-glucopyranosyl)-(1→6)-β-glucopyranosyl ester.

Keywords *Kalopanax pictus*; Araliaceae; harigiri; saponin; kalopanax-saponin; hederagenin glycoside; acetyl-saponin

As a part of our series of studies on araliaceous medicinal plants,[1] we recently reported the isolation and structural determination of three new saponins named kalopanax-saponins C, D and F together with four known saponins from the roots of *Kalopanax septemlobus* (THUNB.) KOIDZ. (Chinese name: ciqiu 刺楸) collected in Jilin district, in the north-east of China. Subsequently, we have also investigated saponins from leaves of the same plant, reporting the isolation and structural determination of three new 22α-hydroxyhederagenin glycosides named kalopanax-saponins La, Lb and Lc, together with five known saponins.[2] *Kalopanax pictus* (THUNB.) NAKAI (Japanese name: harigiri) which grows in Japan, has been considered to be taxonomically identical with the above Chinese plant. Shoji *et al.* have reported the isolation and structural determination of several lignan glycosides and other phenolic glycosides from the bark of the Japanese harigiri.[3] In order to compare the chemical constituents of both the Chinese and Japanese specimens, we have investigated saponins from leaves and roots of Japanese harigiri, *K. pictus* (THUNB.) NAKAI collected in Hiroshima, Japan.

Comparison by thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC) indicated that saponin composition of roots of the Japanese specimen was very similar to that of roots of the Chinese specimen, while the chromatograms of the leaves were found to be somewhat different from each other. The present report deals with the isolation and structural elucidation of two new saponins named kalopanax-saponins JLa (**4**) and JLb (**5**) together with identification of three known saponins from the leaves of Japanese harigiri.

Dried leaves of harigiri were extracted with methanol. A suspension of the methanolic extract in water was washed with ethyl ether and then subjected to chromatography on highly porous polymer. The resulting saponin fraction was separated by repeated chromatography on a silica gel column followed by HPLC to give five saponins, **1**—**5**, in yields of 0.4, 0.2, 0.73, 0.35 and 0.39%, respectively.

Based on analysis of the proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra and the results of acid hydrolysis as well as comparison of the optical rotations, **1** and **2** were proved to be identical with kalopanax-saponins A and B (Chart 1), which have already been isolated from roots and leaves of Chinese Ciqiu.[1,2]

Compound **3** afforded hederagenin (**6**), glucose, rhamnose and arabinose on acid hydrolysis. The ^{13}C- and ^{1}H-NMR spectra (Table I) indicated that **3** has an acetyl group (^{13}C-NMR δ: 170.6 and 20.6; ^{1}H-NMR δ: 1.93 (3H, s)). It is well-known that an acyl linkage on a sugar moiety of a bisdesmosidic saponin is highly unstable, being saponified even under mildly alkaline conditions without any cleavage of the relatively hindered glycosyl ester linkage. On mild alkaline hydrolysis,[4] **3** afforded a deacetyl product which was identified as **2**. On comparison of the ^{13}C-NMR spectrum (Table I) of **3** with that of **2**, on going from **2** to **3**, the signals due to C-6 and C-5 of the central glucose moiety were displaced downfield by 1.9 ppm and upfield by 3.3 ppm, respectively, while other signals remained almost unshifted. This indicated that an acetyl group was located at the 6-hydroxyl group of the central glucosyl unit of the 28-sugar moiety. Based on the above evidence and comparison of the ^{13}C-NMR data with reference values,[5] **3** was concluded to be identical with kizuta saponin K_{11} which has already been obtained from *Hedera rhombea* BEAN[4] (Chart 1).

The ^{13}C-NMR spectra of two new saponins named kalopanaxsaponins JLa (**4**) and JLb (**5**) indicated that both were bisdesmosides of **6** (Table I) having five monosaccharide units. On acid hydrolysis, both **4** and **5** afforded **6**, glucose, rhamnose and arabinose.

The ^{1}H-NMR signals of **4** (δ 1.99, 2.02, (each 3H, s)) and **5** (δ 1.94, 2.02 (each 3H, s)) as well as the ^{13}C-NMR signals of **4** (δ 20.9, 21.1, 170.9 and 171.2) and **5** (δ 20.7, 21.2, 170.7 and 171.0) revealed the presence of two acetyl groups in both saponins. On mild alkaline hydrolysis, **4** and **5** afforded the same desacetyl-saponin, which was identified as **2** by comparison of the physical properties and ^{13}C-NMR spectra. On alkaline hydrolysis under stronger conditions, both **4** and **5** gave the same monodesmosidic prosapogenin, which was identified as **1**. These results indicated that **4** and **5** must be isomers of the diacetate of **2**.

The location of each acetyl group was revealed as follows. The fast atom bombardment mass spectra (FAB-MS) (negative) of both **4** and **5** exhibited the following fragment ions (Chart 2) at *m/z* 1303 $[M-H]^-$, 1157 $[M-Rha-H]^-$, 1025 $[M-(Rha-Ara)-H]^-$, indicating the presence of a terminal rhamnoside unit and an arabinosyl-

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rhamnoside unit, both of which have no acetyl group. The fragment ions at *m/z* 1115 $[M-(Rha-Ac)-H]^-$, 911 $[M-((Rha-Glc)Ac_2)-H]^-$, 749 $[M-((Rha-Glc-Glc)Ac_2)-H]^-$ of both saponins showed that one of the two acetyl groups must be located at the terminal rhamnoside unit and another at the central glucoside unit of the 28-Rha–Glc–Glc moiety of **2**. In the ^{13}C-NMR, on going from **2** to **4**, the signals due to C-6 and C-5 at the central glucosyl unit of the C-28 sugar moiety were displaced downfield by 1.9 and 3.1 ppm, respectively, showing that one of the two acetyl groups must be located on the 6-hydroxyl of the central glucosyl unit, as in the case of **3**. On going from **3** to **4**, signals due to the anomeric carbon (δ 99.4) and C-3 (δ 70.3) of the terminal rhamnosyl unit were displaced upfield by 3.5 and 2.3 ppm, respectively and the signal due to C-2 of this rhamnoside was displaced downfield by 1.7 ppm (acylation shift),[5] while other signals remained almost unshifted, indicating that another acetyl group must be located on the 2-hydroxyl group of the terminal rhamnosyl unit of the C-28 sugar moiety. Base on these results, **4** can be formulated as 3-*O*-α-rhamnopyranosyl-(1→2)-α-arabinopyranosyl hederagenin 28-*O*-(2-*O*-acetyl-α-rhamnopyranosyl)-(1→4)-(6-*O*-acetyl-β-glucopyranosyl)-(1→6)-β-glucopyranosyl ester (Chart 1).

By comparison of the ^{13}C-NMR spectrum (Table I) of **5** with those of **2**, **3** and **4**, it was revealed that one of the two acetyl groups must be at the same position as in **3**. In contrast to the case of **4**, the signals due to five anomeric carbons of **5** appeared at almost the same positions as those of **2** and **3**. On going from **3** to **5**, the signal due to C-3 of the rhamnosyl unit was displaced downfield by 3.6 ppm and the signals due to C-2 and C-4 of the same rhamnosyl unit were displaced upfield by 2.4 and 2.9 ppm (acylation shift), respectively, while other signals remained almost unshifted, indicating that the other acetyl group of **5** must be located at the 3-hydroxyl group of the rhamnosyl unit. These results led to the formulation of **5** as 3-*O*-α-rhamnopyranosyl-(1→2)-α-arabinopyranosyl hederagenin 28-*O*-(3-*O*-acetyl-α-rhamnopyranosyl)-(1→4)-(6-*O*-acetyl-β-glucopyranosyl)-(1→6)-β-glucopyranosyl ester.

It has been reported that acyl groups on *cis* 2- and 3-hydroxyl groups of rhamnoside readily undergo acyl migration. According to the reported method,[6] solutions of **4** and **5** in C_5D_5N were each heated at 80 °C for 60 h, affording an equilibrated mixture which was composed of nearly equal amounts of **4** and **5**. This observation supported the above structures of **4** and **5**.

Previously, we reported the isolation of three 22α-hy-

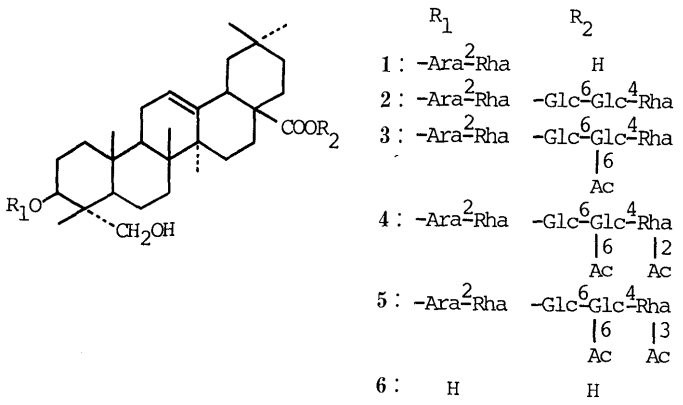

Chart 1. Saponins from Leaves of *Kalopanax pictus*

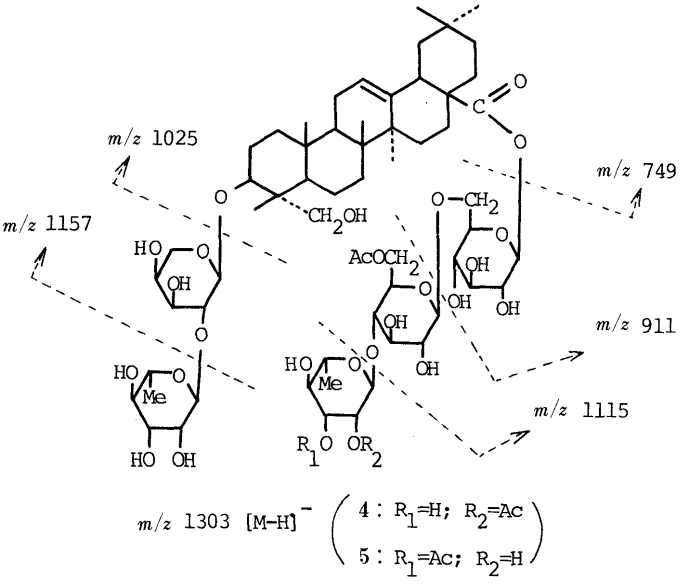

Chart 2. The Fragment Ions of Saponins **4** and **5** Found by FAB-MS (Negative)

TABLE I. ^{13}C-NMR Data for Saponins from Leaves of *K. pictus* in C_5D_5N

	5	4	3	2	1
C-3	81.1	81.2	81.1	81.0	81.1
C-23	64.1	64.2	64.0	64.2	63.9
C-28	176.6	176.7	176.5	176.5	180.1
3-*O*-Sugar moieties					
Ara 1	104.2	104.2	104.2	104.2	104.2
2	75.9	76.3	75.8	75.8	75.8
3	74.4	74.2	74.5	74.1	74.5
4	69.2	69.2	69.2	69.2[a]	69.2
5	65.4	65.4	65.4	65.5	65.5
Rha 1	101.7	101.7	101.6	101.6	101.6
2	72.3[b]	72.3[b]	72.3[b]	72.3[b]	72.3[a]
3	72.5[b]	72.5[b]	72.6[b]	72.5[b]	72.5[a]
4	74.1	74.0	74.0[c]	74.1[c]	74.0
5	69.5[a]	69.8[a]	69.7[a]	69.7[a]	69.6
6	18.6	18.6	18.5	18.5	18.5
28-*O*-Sugar moieties					
Glc 1	95.6	95.6	95.5	95.6	
2	73.9	74.0	73.8[c]	73.9[c]	
3	78.7	78.7	78.6	78.3	
4	70.9	70.8	70.9	70.8	
5	78.0	78.2	78.0	78.0	
6	69.8[a]	69.8[a]	69.7[a]	69.2[a]	
Glc 1	104.8	104.6	104.6	104.8	
2	75.2	75.3	75.0	75.3	
3	76.2	76.3	76.3	76.5	
4	78.8	78.9	79.1	78.6	
5	73.7	74.0	73.8[c]	77.1	
6	63.6	63.6	63.6	61.7	
Rha 1	102.7	99.4	102.9	102.7	
2	69.9[a]	74.0	72.3[b]	72.5[b]	
3	76.2	70.3[b]	72.6[b]	72.7[b]	
4	70.9[c]	74.0	73.8[c]	73.8[c]	
5	70.7[c]	70.5[b]	70.6	70.3	
6	18.6	18.6	18.5	18.5	
CH_3CO	171.0	171.2	170.6		
	170.7	170.9			
CH_3CO	20.7	20.9	20.6		
	21.2	21.1			

a—*c*) Assignments may be interchanged in each column.

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droxyhederagenin glycosides from the leaves of Ciqiu collected in China.[2] However, in the present study, no saponin of 22-α-hydroxyhederagenin has been isolated from leaves of Japanese harigiri, while the acylsaponins, **3**, **4** and **5** were hardly detected in leaves of Chinese Ciqiu.

Experimental

Melting points were measured on a micro hot-stage and are uncorrected. Optical rotations were taken on a Union PM-101 automatic digital polarimeter. NMR spectra were recorded on a JEOL GX-400 spectrometer in C_5D_5N solution using tetramethylsilane (TMS) as an internal standard. For gas-liquid chromatography (GLC), a Shimadzu GC-6A apparatus was used. MS were taken on a JEOL JMS-SX 102 spectrometer. HPLC was carried out on a column of TSK-gel ODS-120T (21.5 mm × 30 cm) with a Toyo Soda HLC 803D pump and a Toyo Soda RI-8 differential refractometer for detection. For column chromatography, Kieselgel 60 (70—230 mesh, Merck), Diaion HP-20 (Mitsubishi Chem. Ind. Co., Ltd.) and Polyamide C-200 (Wakojunyaku Ind. Co., Ltd. 74—420 μ) were used. All solvent systems for chromatography were homogeneous.

Extraction and Separation of Saponins Leaves of *Kalopanax pictus* (THUNB.) NAKAI (harigiri) were collected at the Experimental Station of Medicinal Plants, Hiroshima University, School of Medicine (June, 1989). The dried leaves (100 g) were extracted with hot MeOH. A suspension of the MeOH extract (15 g) in H_2O was washed with Et_2O. The H_2O layer, after removal of residual ether, was chromatographed on a column of highly porous polymer (Diaion HP-20) and eluted with H_2O and MeOH, successively. The MeOH fraction (10.5 g) was subjected to chromatography on silica gel. Elution with $CHCl_3$–MeOH–H_2O (30 : 10 : 1) provided five fractions (fr. A, B, C, D and E in increasing order of *Rf* on silica gel TLC, developed with $CHCl_3$–MeOH–H_2O (14 : 6 : 1)). Fraction D (1.5 g) was crystallized from MeOH to give **1** (40 mg). Fraction A (150 mg) was purified by HPLC (ODS-120T column) with 65% MeOH (flow rate, 6 ml/min) to give **2** (24 mg). Fraction B (758 mg) was chromatographed on a polyamide column with 30% MeOH, and the 30% MeOH eluate was purified by HPLC with 65% MeOH to give **3** (733 mg). Fraction C (1.85 g) was chromatographed on polyamide with 30% MeOH, and then the 30% MeOH eluate was further separated by HPLC With 65% MeOH to afford **4** (352 mg) and **5** (389 mg). **1**: Colorless needles (MeOH), mp 249—251 °C (dec.). $[\alpha]_D^{24}$ +17.5° (c= 0.50, MeOH). **2**: A white powder, $[\alpha]_D^{24}$ − 8.0° (c=0.50, MeOH). **3**: A white powder, $[\alpha]_D^{24}$ −21.5° (c=0.70, MeOH). ^{1}H-NMR δ: 0.87, 0.90, 0.98, 1.07, 1.11, 1.17 (each 3H, s), 1.62, 1.69 (each 3H, d, J=6 Hz, Me of Rha), 1.93 (3H, s, CH_3CO), 4.99 (1H, d, J=7.5 Hz, anomeric H), 5.10 (1H, d, J=6.7 Hz, anomeric H), 5.39 (1H, br s, 12-H), 5.52 (1H, s, anomeric H of Rha), 6.20 (anomeric H, overlapping), 6.22 (1H, s, anomeric H of Rha). FAB-MS (negative) m/z: 1261 $[M-H]^-$, 1115 $[M-Rha-H]^-$, 911 $[M-((Rha-Glc)Ac)-H]^-$, 749 $[911-Glc]^-$, 471 $[749-(Rha-Ara)]^-$.

Compound **4**: A white powder, $[\alpha]_D^{24}$ −19.1° (c=0.89, MeOH). *Anal.* Calcd for $C_{63}H_{100}O_{28}\cdot H_2O$: C, 57.17; H, 7.77. Found: C, 57.46; H, 8.15. ^{1}H-NMR δ: 0.90, 0.93, 0.98, 1.07, 1.11, 1.17 (each 3H, s), 1.64, 1.74 (each 3H, d, J=5.9 Hz, Me of Rha), 4.98, 5.07 (each 1H, d, J=7 Hz, anomeric H), 5.38 (1H, br s, 12-H), 5.50 (1H, s, anomeric H of Rha), 6.22 (2H, anomeric H, overlapping). FAB-MS (negative) m/z: 1303 $[M-H]^-$, 1157 $[M-Rha-H]^-$, 1115 $[M-((Rha)Ac)-H]^-$, 1025 $[M-(Rha-Ara)-H]^-$, 911 $[M-((Rha-Glc)Ac_2)-H]^-$, 749 $[911-Glc]^-$, 603 $[749-Rha]^-$, 471 [aglycone − H]$^-$.

Compound **5**: A white powder, $[\alpha]_D^{24}$ −12.4° (c=0.47, MeOH). *Anal.* Calcd for $C_{63}H_{100}O_{28}\cdot H_2O$: C, 57.17; H, 7.77. Found: C, 57.23; H, 8.03. ^{1}H-NMR δ: 0.90 (6H, s), 0.98, 1.07, 1.12, 1.17 (each 3H, s), 1.64, 1.72 (each 3H, d, J=5.9 Hz, Me of Rha), 1.94, 2.02 (each 3H, s, CH_3CO), 4.95 (1H, d, J=6 Hz, anomeric H), 5.42 (1H, br s, 12-H), 5.74 (1H, d, J= 7 Hz, anomeric H), 5.53 (1H, s, anomeric H of Rha), 6.23 (1H, s, anomeric H of Rha), 6.25 (anomeric H, overlapping). FAB-MS (negative) m/z: 1303, 1157, 1115, 1025, 911, 749, 603, 471.

Acid Hydrolysis of 1—5 A solution of saponin (1—2 mg) in 7% HCl–dioxane (1 : 1) (2 ml) was heated at 90 °C for 3 h, according to the method reported previously. Compound **1**—**5** afforded the same aglycone (**6**), and **1** also afforded arabinose and rhamnose, while **2**—**5** afforded glucose, arabinose and rhamnose.

Alkaline Hydrolysis of 3, 4 and 5 Compound **4** (or **3** and **5**) (40 mg) was hydrolyzed by heating in aqueous 0.5 N KOH (4 ml) at 90 °C for 1 h. The reaction mixture was neutralized with Amberlite MB-3 resin and then partitioned between H_2O and BuOH. The BuOH layer was concentrated to dryness to give **1**, which was identified on the basis of TLC behavior, and ^{1}H- and ^{13}C-NMR spectra.

Deacetylation of 3, 4 and 5 A solution of **4** (or **3** and **5**) (40 mg) in aqueous 0.05 N KOH (3 ml) was allowed to stand at 4 °C for 24 h. The reaction mixture was neutralized with Amberlite MB-3 resin, and then the mixture was extracted with BuOH and the BuOH layer was concentrated to dryness to give **2** (30 mg).

Acyl Migration of 4 and 5[6] A solution of **4** (40 mg) in C_5D_5N was heated at 80 °C for 60 h. The ^{13}C-NMR spectrum of the reaction product showed that it is a mixture of nearly equal amounts of **4** and **5**. Similar acyl migration was also observed in the case of **5**, which was treated similarly.

References

1) C.-J. Shao, R. Kasai, J.-D. Xu and O. Tanaka, *Chem. Pharm. Bull.*, **37**, 311 (1989).
2) C.-J. Shao, R. Kasai, K. Ohtani, J.-D. Xu and O. Tanaka, *Chem. Pharm. Bull.*, **12**, 3251 (1989).
3) K. Sano, S. Sanada, Y. Ida and J. Shoji, The Abstracts Book of 31st Annual Meeting of the Japanese Society of Pharmacognosy, Tokyo, p. 74, 1984.
4) H. Kizu, S. Hirabayashi, M. Suzuki and T. Tomimori, *Chem. Pharm. Bull.*, **33**, 3473 (1985).
5) K. Yamasaki, R. Kasai, Y. Masaki, M. Okihara, O. Tanaka, H. Oshio, S. Takagi, M. Yamaki, K. Masuda, G. Nonaka, M. Tsuboi and I. Nishioka, *Tetrahedron Lett.*, **1977**, 1231; K. Yoshimoto, Y. Itatani and Y. Tsuda, *Chem. Pharm. Bull.*, **28**, 2065 (1980).
6) T. Konishi, A. Tada, J. Shoji, R. Kasai and O. Tanaka, *Chem. Pharm. Bull.*, **26**, 668 (1978); S. Asen and R. M. Horowitz, *Phytochemistry*, **16**, 147 (1977); V. M. Chari, M. Jordan, H. Wagner and P. W. Thies, *ibid.*, **16**, 1110 (1977).

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