Saponins from Leaves of *Kalopanax septemlobus* (THUNB.) KOIDZ.: Structures of Kalopanax-saponins La, Lb and Lc

Chun-Jie SHAO,^a Ryoji KASAI,^b Kazuhiro OHTANI,^b Jing-Da XU,^a and Osamu TANAKA^{*,b}

Department of Chemistry, Bethune University of Medical Sciences,^a Xinmin Street 6, Changchun, China and Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine,^b Kasumi, Minami-ku, Hiroshima 734, Japan. Received June 23, 1989

From leaves of *Kalopanax septemlobus* (THUNB.) KOIDZ., three new saponins named kalopanax-saponins La (6), Lb (7) and Lc (8) were isolated together with five known saponins, kalopanax-saponins A and B, akeboside Stb, eleutheroside K and saponin Pg. The new sapogenin 9 named kalopanax-genin L1, which is common to 6, 7 and 8, was formulated as 22 α -hydroxyhederagenin. On the basis of chemical and spectral data, the structures of these saponins were elucidated as follows: 3-*O*- α -L-arabinopyranosyl-22 α -hydroxyhederagenin (6), 3-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranosyl-22 α -hydroxyhederagenin (7) and 3-*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranosyl-22 α -hydroxyhederagenin (8).

Keywords *Kalopanax septemlobus*; Araliaceae; Chinese folk medicine; ciqui; saponin; kalopanax-saponin; 22 α -hydroxyhederagenin; 3 β ,22 α ,23-trihydroxyolean-12-en-28-oic acid

In the previous paper,¹⁾ we reported the isolation and structural determination of three new saponins named kalopanax-saponins C, D and F²⁾ together with four known saponins from roots of *Kalopanax septemlobus* (THUNB.) KOIDZ (Chinese name: ciqui 刺楸, Japanese name: harigiri) collected in Jilin district, North-East China. Continuing the investigation of this plant, we have studied saponins of the leaves of this plant. The present report deals with isolation and structure elucidation of three new saponins together with identification of five known saponins from the leaves.

Air-dried leaves of *Kalopanax septemlobus* were extracted with methanol. A suspension of the methanolic extract in water were washed with ethyl ether and extracted with ethyl acetate and then with 1-butanol saturated with water, successively. The ethyl acetate extract was subjected to chromatography on silica gel followed by high-performance liquid chromatography (HPLC) to give saponins 1, 2, 3, 4, 6, 7 and 8 (yields from dried leaves: 0.008, 0.01, 0.21, 0.006, 0.01, 0.002 and 0.04%, respectively). Chromatography of the butanolic extract in a similar manner afforded a saponin 5 (yield from dried leaves:

0.05%).

Based on the proton- and carbon-13 nuclear magnetic resonance (¹H- and ¹³C-NMR) evidence and the result of acid hydrolysis, as well as comparison of the optical rotations, saponin 1 was shown to be identical with akeboside Stb from *Akebia quinata* (= β 2-fatsin from *Fatsia japonica*).³⁾ In the same manner, saponin 2 was found to be identical with eleutheroside K from *Acanthopanax senticosus*⁴⁾ and saponins 3 and 5 were proved to be identical with kalopanax-saponins A and B,^{1,5)} respectively. Saponin 4 was identical with saponin Pg from *Akebia quinata*.⁶⁾

A new saponin 6 named kalopanax-saponin La, on acid hydrolysis, afforded arabinose and a new sapogenin 9 named kalopanax-genin L1. The positive and negative fast atom bombardment mass spectrum (FAB-MS) of 9 exhibited (M + Na)⁺ at *m/z* 511 (488 + 23) and (M - H)⁻ at *m/z* 487 (488 - 1), respectively. The ¹H- and ¹³C-NMR spectra (in C₅D₅N) of 9 showed six quaternary methyl signals [proton signals at δ 0.93, 0.94, 1.10, 1.11, 1.12, 1.14 (each

Chart 1

Chart 2

TABLE I. ^{13}C -NMR Chemical Shifts of Kalopanax Saponins in $\text{C}_5\text{D}_5\text{N}$

	Aglycone moieties						Sugar moieties		
	9	11 ^{a)}	6	7	8	10 ⁷⁾	6	7	8
							Ara		
C-1	38.80	37.74	38.79	38.65	39.23	38.53	1	106.63	104.25
C-2	27.97	23.40	26.10	26.22	26.37	27.40	2	73.14	75.88
C-3	73.59	74.51	81.91	81.05	81.44	73.32	3	74.68	74.32
C-4	42.60	40.53	42.56	42.55	42.89	42.59	4	69.58	69.16
C-5	48.64	47.61	47.61	47.67	48.01	48.48	5	66.92	65.36
C-6	18.62	17.93	18.19	18.52	18.47	18.34	Rha		
C-7	32.85	32.16	32.77	32.80	33.04	32.74	1		101.67
C-8	39.96	39.43	40.02	39.96	40.16	39.54	2		72.52
C-9	48.15	47.86	48.15	48.15	48.39	47.92	3		72.38
C-10	37.19	36.72	36.94	36.85	37.14	37.00	4		74.13
C-11	23.88	22.93	23.89	23.75	23.93	23.50	5		69.30
C-12	122.82	123.27	122.88	122.77	122.62	122.23	6		18.52
C-13	144.21	142.17	144.19	144.26	144.51	144.57	Xyl		
C-14	42.09	41.69	42.56	42.55	42.88	41.95	1		107.17
C-15	27.69	26.86	27.98	27.98	28.27	28.10	2		75.54
C-16	16.90	16.82	16.90	17.01	17.49	23.45	3		78.27
C-17	53.08	50.99	53.10	53.08	53.17	46.21	4		71.16
C-18	43.52	42.39	43.53	43.52	43.67	41.75	5		67.31
C-19	46.20	44.90	45.95	45.98	46.70	46.40			
C-20	31.53	31.42	31.53	31.48	31.58	30.67			
C-21	43.23	39.10	43.28	43.52	43.77	33.96			
C-22	71.54	72.72	71.52	71.55	71.94	32.96			
C-23	67.99	65.45	64.53	64.50	64.53	67.92			
C-24	13.11	13.07	13.59	13.99	14.04	12.78			
C-25	15.94	15.74	16.07	16.03	16.28	15.69			
C-26	17.50	16.75	17.44	17.45	17.79	17.23			
C-27	26.71	26.23	26.71	26.71	26.66	25.88			
C-28	179.50	175.04	179.40	179.60	180.05	179.86			
C-29	33.39	32.88	33.38	33.39	33.53	32.96			
C-30	25.15	24.67	25.16	25.20	25.29	23.59			
OCH ₃		51.94							
CH ₃ CO		170.94							
		170.69							
		170.13							
CH ₃ CO		21.21							
		21.01							
		20.90							

a) Determined in CDCl_3 .

3H, s), carbon signals see Table I] and exhibited signals due to the following groups at very similar positions to those observed for hederagenin (**10**)⁷⁾: a carboxyl group (a carbon signal at δ 179.5), a trisubstituted double bond [carbon signals at δ 122.8 and 144.2, a proton signal at δ 5.51 (1H, t-like)], a primary alcoholic group [a carbon signal at δ 68.0, proton signals at δ 3.75, 4.20 (each 1H, d, $J=10.8$ Hz)] and a secondary alcoholic group [a carbon signal at δ 73.6, a proton signal at δ 4.25 (1H, dd, coupling constant could not be determined due to partial signal overlap)]. A proton signal due to 18-H which is characteristic of olean-12-en-28-oic acid type triterpenes⁷⁾ was observed for **9** at δ 3.56 (1H, dd, $J=4.4$, 10.2 Hz) and the presence of an additional secondary hydroxyl group was indicated by a carbon signal at δ 71.5 and a proton signal at δ 4.58 (1H, dd, $J=5.9$, 10.8 Hz). The ^1H -NMR spectrum (in CDCl_3) of a methyl ester acetate (**11**) of **9** showed signals due to six quaternary methyls [δ 0.73, 0.87, 1.09, 1.17 (each 3H, s), 0.99 (6H, s)], three acetoxyl groups [δ 2.04, 2.06, 2.16 (each 3H, s)], a carbomethoxy group [δ 3.62 (3H, s)], the 18-H [δ 2.99 (1H, dd, $J=4.4$, 10.3 Hz)], a primary acetoxyl group [δ 3.73, 3.92 (each 1H, d, $J=11.6$ Hz)] and two secondary acetoxyl groups [δ 4.82 (1H, dd, $J=5.1$, 11.2 Hz), 5.22 (1H, dd, $J=$

7.0, 10.3 Hz)]. The fragment-ions which are characteristic of the retro Diels-Alder cleavage of olean-12-en-28-oic acid derivatives were observed as follows (Chart 2); the positive FAB-MS of **9** showed ions at m/z 223 ($a+H$)⁺ and 265 ($b+H$)⁺ and the electron impact mass spectrum (EI-MS) of a methyl ester (**12**) of **9** exhibited ions at m/z 278 (b'), 219 ($b'-\text{COOCH}_3$) and 201 ($219-\text{H}_2\text{O}$). These results suggested that **9** is a hydroxyhederagenin with an additional hydroxyl group located in the D or E ring.

In the ^{13}C -NMR (Table I), on going from **10** to **9**, signals due to 17-, 21- and 22-C were displaced downfield by 6.9, 9.2 and 38.6 ppm, respectively and that due to 16-C was significantly shifted upfield by 6.6 ppm, while other carbon signals remained at almost unchanged positions. Similar displacement of carbon signals was reported from erythrodol (olean-12-ene-3 β ,28-diol) to 22 α -hydroxyerythrodol.⁸⁾ Taking into account the hydroxylation shift rule and the γ -gauche effect⁹⁾ as well as the coupling constants of the carbonyl proton signal of **9** (and **11**) (*vide supra*), it follows that the additional secondary hydroxyl group of **9** must be located at 22 α (equatorial). Assignment of proton signals due to the 29- and 30-*gem*-dimethyl groups of **11** was substantiated by ^1H - ^1H and ^1H - ^{13}C -correlation spec-

troscopy (COSY) as well as by the COLOC procedure. Irradiation of a signal at δ 1.09 (3H, s, 30-CH₃)¹⁰ resulted in nuclear Overhauser effect (NOE) at signals due to 18-H (5.5%) and 22 β -H (4.4%), confirming the location of the secondary hydroxyl group at 22 α . Based on these results, **9** can be formulated as 22 α -hydroxyhederagenin.

The positive FAB-MS of **6** showed ions at m/z 643 (M+Na)⁺, 621 (M+H)⁺, 489 (M+H-Ara)⁺. On comparison of the ¹³C-NMR spectrum of **6** with that of **9** (Table I), the signal ascribable to 3-C of the aglycone moiety was displaced downfield by 8.3 ppm and the signal due to 2-C was displaced upfield by 1.9 ppm (glycosylation shift),¹¹⁻¹³ while other signals remained almost unshifted. Further, signals due to the sugar moiety of **6** (Table I) appeared at almost the same positions as those of α -L-arabinopyranosides¹³ such as **1**.¹¹ It follows that **6** can be formulated as the 3-*O*- α -L-arabinopyranoside of **9**, as shown in Chart 1.

A new saponin **7** named kalopanax-saponin Lb, exhibited ions at m/z 789 (M+Na)⁺ and 767 (M+H)⁺ in the positive FAB-MS and on acid hydrolysis, **7** afforded **9**, arabinose and rhamnose. Comparison of the ¹³C-NMR spectrum of **7** with that of **9** demonstrated the same glycosylation shift around 3-C as observed for **6** (Table I). Carbon signals of **7** at δ 104.3 and 101.7 indicated the presence of two sugar units. The observation of a fragment ion at m/z 621 (M+H-Rha)⁺ in the positive FAB-MS of **7** indicated the presence of a terminal rhamnose unit. Carbon signals due to the sugar moiety of **7** (Table I) appeared at almost the same positions as those of 2-*O*- α -L-rhamnopyranosyl- α -L-arabinopyranosides¹⁴ such as **3**.¹¹ Based on these results, **7** can be formulated as the 3-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranoside of **9**.

Another new saponin **8**, named kalopanax-saponin Lc, exhibited ions at m/z 921 (M+Na)⁺ and 899 (M+H)⁺ in the positive FAB-MS and afforded **9**, arabinose, rhamnose and xylose on acid hydrolysis. The glycosylation shift around 3-C as well as three anomeric carbon signals at δ 107.2, 104.3 and 101.5 in the ¹³C-NMR spectrum of **8** (Table I) disclosed that **8** is a 3-*O*-glycoside of **9** which has three monosaccharide units. In the positive FAB-MS of **8**, ions at m/z 767 [M+H-pentose]⁺, 621 [M+H-pentose-Rha]⁺ and 489 [M+H-pentose-Rha-pentose]⁺ indicated that the sugar moiety of **8** consists of a linear pentose-rhamnose-pentose unit. On partial hydrolysis, **8** afforded **6** and **7** which were identified by comparison of the ¹H- and ¹³C-NMR spectra with those of respective authentic samples. Further, carbon signals due to the sugar moiety of **8** (Table I) were observed at almost the same positions as those of **4**.¹¹ From these results, the structure of **8** was established as the 3-*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -arabinopyranoside of **9**.

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. Infrared (IR) spectra were taken on a Shimadzu FTIR-4200 spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL GX-400 spectrometer using tetramethylsilane (TMS) as an internal standard. MS were taken on a JEOL JMS-SX 102 spectrometer. Preparative HPLC was carried out on a column of TSK-gel ODS-120T (21.5 mm i.d. $\times$ 30 cm) with a Toyo Soda HLC 803D pump and a Toyo

Soda RI-8 differential refractometer as a detector (flow rate of mobile phase: 6 ml/min). For column chromatography, Kieselgel 60 (70–230 mesh, Merck) was used. All solvent systems for chromatography were homogeneous.

Extraction and Separation of Saponins The air-dried leaves of *Kalopanax septemlobus* (1.5 kg), collected in Jilin, China, was extracted with hot MeOH. A suspension of the MeOH extract (139.5 g) in H₂O was washed with Et₂O and extracted with AcOEt and then with 1-BuOH saturated with H₂O. The AcOEt layer and BuOH layer were each concentrated to dryness to give the AcOEt extract (34 g) and BuOH extract (11.4 g), respectively. The AcOEt extract was chromatographed on silica gel first with CHCl₃-MeOH (6:1) and then with CHCl₃-MeOH-H₂O (40:10:1) to give three fractions (frs.), frs. I, II and III in order of elution, and each of these was separated and purified by HPLC: fr. I (mobile phase 85% MeOH) gave **1** (yield from dried leaves: 0.008%) and **2** (0.01%); fr. II (mobile phase 77% MeOH) afforded **3** (0.21%) and **6** (0.006%); fr. III (mobile phase 75% MeOH) gave **4** (0.01%), **7** (0.002%) and **8** (0.004%). The BuOH extract was chromatographed on silica gel with CHCl₃-MeOH-H₂O (30:10:1 and 70:30:5, successively) to give frs. IV and V, the latter was purified by HPLC with 60% MeOH to give **5** (yield, 0.05%).

1: Colorless needles (MeOH), mp 228–230 °C (dec.), $[\alpha]_D^{20} + 48.0^\circ$ ($c = 0.60$, MeOH).

2: A white powder, $[\alpha]_D^{20} + 11.3^\circ$ ($c = 0.52$, MeOH).

3: Colorless needles (MeOH), mp 250–251 °C, $[\alpha]_D^{20} + 16.8^\circ$ ($c = 0.50$, MeOH).

4: A white powder, $[\alpha]_D^{20} + 9.6^\circ$ ($c = 0.46$, MeOH).

5: A white powder, $[\alpha]_D^{20} - 8.3^\circ$ ($c = 0.80$, MeOH).

6: A white powder, $[\alpha]_D^{20} + 40.4^\circ$ ($c = 0.42$, MeOH). *Anal.* Calcd for C₃₅H₅₆O₉·H₂O: C, 65.80; H, 9.15. Found: C, 65.75; H, 9.12. ¹H-NMR (C₅D₅N) δ : 0.93, 0.94, 1.00, 1.07, 1.12, 1.29 (each 3H, s), 5.49 (1H, t-like, 12-H), 4.99 (1H, d, $J = 7.0$ Hz, anomeric H).

7: A white powder, $[\alpha]_D^{20} + 49.3^\circ$ ($c = 0.55$, MeOH). *Anal.* Calcd for C₄₁H₆₆O₁₃·2H₂O: C, 61.32; H, 8.79. Found: C, 61.42; H, 8.64. ¹H-NMR (in C₅D₅N) δ : 0.94, 0.99, 1.12, 1.28 (each 3H, s), 1.07 (6H, s), 1.64 (3H, d, $J = 6.0$ Hz, CH₃ of Rha), 5.16 (1H, d, $J = 7.0$ Hz, anomeric H of Ara), 6.27 (1H, s, anomeric H of Rha), 5.48 (1H, t-like, 12-H).

8: A white powder, $[\alpha]_D^{20} + 45.6^\circ$ ($c = 0.40$, MeOH). *Anal.* Calcd for C₄₆H₇₄O₁₇·2H₂O: C, 59.08; H, 8.41. Found: C, 59.49; H, 8.69. ¹H-NMR (C₅D₅N) δ : 0.92, 1.03, 1.06, 1.27 (each 3H, s), 0.97 (6H, s), 1.66 (3H, d, $J = 6.0$ Hz, CH₃ of Rha), 5.01, 5.12 (each 1H, d, $J = 7.0$ Hz, anomeric H of Ara and Xyl), 5.98 (1H, s, anomeric H of Rha), 5.38 (1H, t-like, 12-H).

Acid Hydrolysis of 6, 7 and 8 On heating with 1.5% aqueous H₂SO₄ for 24 h at 70 °C followed by work-up in the usual way, **6** afforded **9** and arabinose; **7** afforded arabinose, rhamnose and **9**; **8** afforded arabinose, rhamnose, xylose and **9**. Identification of the resulting monosaccharides was carried out as described in the previous paper.¹⁵

9: A white powder, $[\alpha]_D^{20} + 67.3^\circ$ ($c = 0.32$, EtOH). *Anal.* Calcd for C₃₀H₄₈O₅·2H₂O: C, 68.67; H, 9.99. Found: C, 68.70; H, 9.77. IR (Nujol) cm⁻¹: 3360 (OH), 1696 (COOH).

Methyl Ester Acetate (11) of 9 The aglycone **9** (20 mg) was treated with CH₂N₂ in MeOH-Et₂O and the product was purified by column chromatography on silica gel [solvent: CHCl₃-MeOH (10:1)] to give a methyl ester (**12**) as a white powder, which was acetylated by heating with anhydrous (CH₃CO)₂O (1.5 ml) and C₅H₅N (1.5 ml) in a sealed tube at 80 °C for 2–3 h. The reaction mixture was concentrated to dryness by blowing N₂ gas over it at room temperature and then purified by column chromatography on silica gel [solvent: benzene-acetone (8:1)] to give **11** (15 mg) as a white powder. Positive FAB-MS (M+H)⁺ Calcd for C₃₇H₅₆O₈ + H: 629.4053; Found: 629.4027.

Partial Hydrolysis of 8 A solution of **8** (45 mg) in aqueous 1.5% H₂SO₄ (15 ml) was heated at 70 °C for 8 h. The reaction mixture was diluted with H₂O and then extracted with 1-BuOH saturated with H₂O. The BuOH layer was concentrated to dryness. The residue was chromatographed on silica gel with CHCl₃-MeOH (4:1) to give **6** (10 mg) and **7** (12 mg), identification of which was achieved by comparison of the ¹H- and ¹³C-NMR spectra with those of respective authentic samples.

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References and Notes

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