Studies on the Constituents of the Bark of *Kalopanax pictus* NAKAI[1)]

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Five new compounds, kalopanaxsaponin G (2) and kalopanaxins A (6), B (8), C (11) and D (13), were isolated from the bark of *Kalopanax pictus* together with nine known compounds, kalopanaxsaponins A (1) and B (5), pericarpsaponin P_{J3} (3), hederasaponin B (4), syringin (7), protocatechuic acid (9), coniferin (10), liriodendrin (=*dl*-syringaresinol di-*O*-glucopyranoside) (12), glucosyringic acid (14) and chlorogenic acid (15). The structures of the new compounds were characterized as hederagenin 28-*O*-α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside (2), ferulylaldehyde (=coniferylaldehyde) 4-*O*-β-D-glucopyranoside (6), coniferin 6′-*O*-(4-*O*-α-L-rhamnopyranosyl)-syringate (8), 2-methoxyhydroquinone 4-*O*-[6-*O*-(4-*O*-α-L-rhamnopyranosyl)-syringyl]-β-D-glucopyranoside (11) and coniferyl alcohol 4-*O*-β-D-apiofuranosyl(1→2)-β-D-glucopyranoside (=coniferin 2′-*O*-β-D-apiofuranoside) (13).

Keywords Araliaceae; *Kalopanax pictus*; saponin; glycoside; triterpenoid; hederagenin; oleanolic acid; kalopanaxin; phenolic compound; phenylpropanoid

The dried bark of *Kalopanax pictus* NAKAI (Japanese name: harigiri) (Araliaceae) has been used as a medicine in China for expelling so-called wind-evil, eliminating wetness, destroying intestinal parasites, promoting blood circulation, and treating paralysis caused by wind and damp, pain along the knee and spine, and pain owing to necrosis and cutaneous fungus.[2)] As for the constituents of this plant, the substances reported so far are as follows: polyacetylenic compounds,[3)] tannin, flavonoid and coumarin glycosides, small amounts of alkaloid, essential oil, resin and starch,[2)] and two hederagenin glycosides, kalopanaxsaponins A and B.[4)] Recently, Shao *et al.* isolated seven triterpenoid saponins including four new ones, namely kalopanaxsaponins C—F, from *K. septemlobus* root collected in China[5a)]: this plant has been thought to be of the same species as *K. pictus* in Japan, as described in their paper. In the course of our phytochemical investigations on Araliaceous plants, we studied the constituents of the bark of *K. pictus* collected in Yamanashi prefecture, Japan. The present paper deals with the isolation and characterization of fifteen compounds from the plant bark.

The fresh bark was extracted with methanol and the extract was treated to afford a saponin fraction (fr-2) and a phenolic fraction (fr-3), and repeated chromatography of the fractions furnished fifteen compounds, **1**—**15**, as described in the experimental section.

Five compounds, **1**—**5**, obtained from fr-2 tested positive in the Liebermann–Burchard reaction and showed strong hydoxyl absorption in the infrared (IR) spectra. Further, the IR spectra of **2**—**5** showed ester absorption (1725 cm^{-1}),

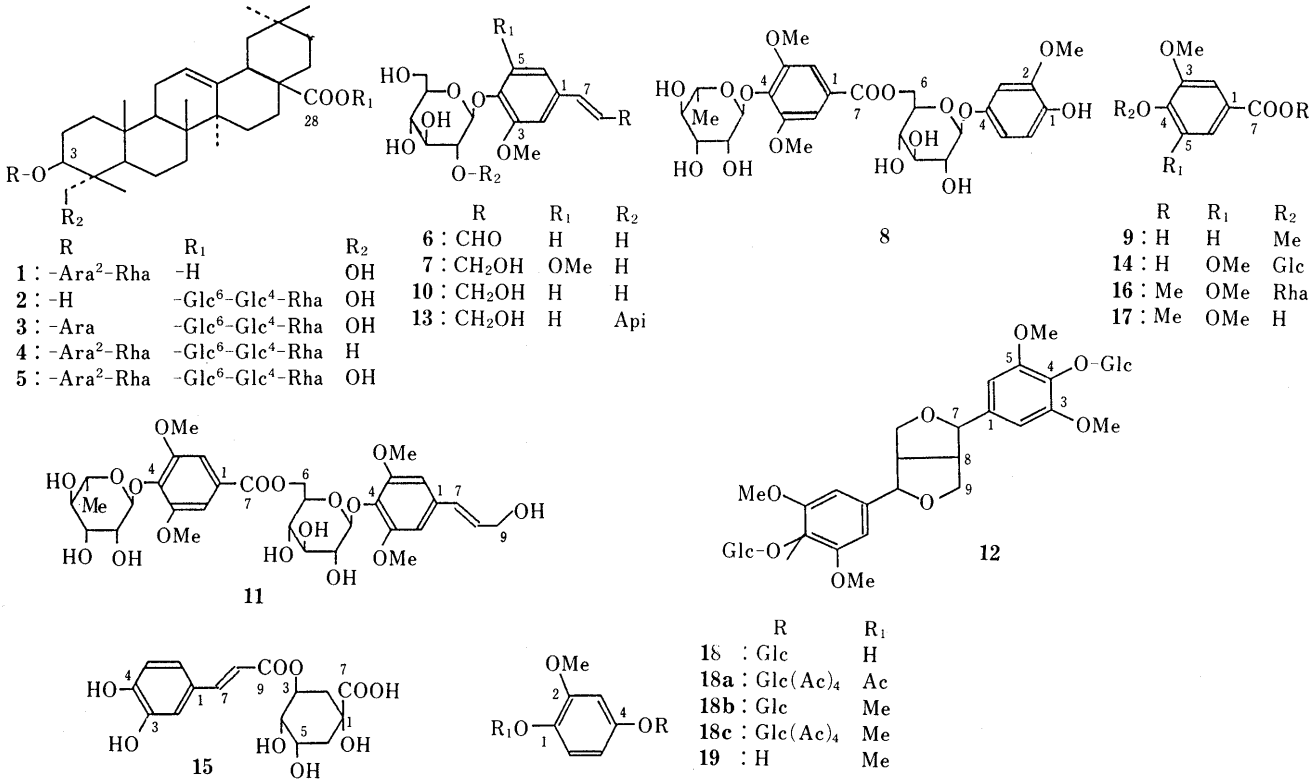

Chart 1. Structures of the Compounds

TABLE I. ^{13}C-NMR Signals of Saponins **1**—**5** in Pyridine-d_5

	1	2	3	4	5
Aglycone					
C-1	39.0	38.9	38.8	38.9	39.1
2	26.2	27.7	26.0	28.1	26.2
3	81.1	73.5	82.0	88.8	81.1
4	43.5	42.9	43.5	39.5	43.5
5	47.8	48.7	47.6	55.9	47.8
6	18.2	18.7	18.2	18.5	18.2
7	32.9	32.6	32.6	32.7	32.6
8	39.8	39.9	39.9	39.9	39.9
9	48.2	48.2	48.2	48.1	48.2
10	36.9	37.3	36.9	37.1	36.9
11	23.8	23.8	23.7	23.7	23.7
12	122.6	122.9	122.9	122.4	122.9
13	144.8	144.1	144.1	144.1	144.1
14	42.2	42.2	42.1	42.2	42.2
15	28.4	28.4	28.3	28.1	28.3
16	23.8	23.5	23.4	23.4	23.4
17	46.7	47.0	47.0	47.1	47.1
18	42.0	41.7	41.7	41.7	41.7
19	46.4	46.2	46.2	46.4	46.2
20	31.0	30.7	30.7	30.8	30.8
21	34.3	34.1	34.0	34.1	34.1
22	33.3	32.9	32.6	33.2	32.8
23	64.1	67.9	64.5	28.1	64.0
24	14.0	13.1	13.6	17.0	14.0
25	16.1	16.1	16.3	15.7	16.2
26	17.4	17.6	17.6	17.5	17.6
27	26.2	26.1	26.0	26.0	26.0
28	180.0	176.5	176.5	176.5	176.5
29	33.3	33.1	33.1	33.2	33.1
30	23.8	23.7	23.7	23.7	23.7

	1	2	3	4	5
Sugars at C-3 of aglycone					
Ara-1	104.3	—	106.6	104.7	104.3[a]
2	75.8	—	73.1	75.9	75.9
3	74.7	—	74.7	73.6	74.6
4	69.3	—	69.6	68.5	69.2
5	65.7	—	67.0	64.7	65.5
Rha-1	101.7	—	—	101.2	101.7
2	72.4	—	—	72.3	72.3[b]
3	72.6	—	—	72.5	72.5[b]
4	74.1	—	—	73.9	74.1
5	69.7	—	—	69.9	69.7
6	18.6	—	—	18.5	18.5
Sugars at C-28 of aglycone					
Glc-1		95.6	95.6	95.6	95.6
2		75.3	75.3	75.3	75.3
3		78.3	78.2	78.3	78.4
4		70.3	70.3	70.3	70.3[c]
5		76.5	76.5	76.5	76.5
6		70.9	70.9	70.9	70.9
Glc-1		104.8	104.8	104.7	104.8[a]
2		73.9	73.9	73.9	74.0
3		78.0	78.1	78.1	78.0
4		78.7	78.7	78.7	78.7
5		77.1	77.1	77.1	77.1
6		61.3	61.3	61.3	61.3
Rha-1		102.7	102.7	102.7	102.7
2		72.5	72.6	72.5	72.5[b]
3		72.7	72.7	72.7	72.5[b]
4		73.8	73.9	73.9	74.0
5		69.2	69.2	69.3	69.2[c]
6		18.5	18.5	18.5	18.5

a—*c*) Assignments with the same superscript in each column may be interchanged.

while that of **1** showed carboxylic absorption (1700 cm^{-1}). On acid hydrolysis, they provided the following aglycones and sugar components: arabinose, rhamnose and hederagenin from **1**; glucose, rhamnose and hederagenin from **2**; arabinose, rhamnose, glucose and hederagenin from **3** and **5**; arabinose, rhamnose, glucose and oleanolic acid from **4**. Their ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectra indicated that there are two sugar units in **1**, three in **2**, four in **3**, and five in **4** and **5** (Table I). Further, the C-3 signal of **1**, **3**, **4** and **5** appeared at lower field and the C-28 signal of **2**, **3**, **4** and **5** at higher field than those of oleanolic acid, because of glycosylation shifts. Hence, they were deduced to be hederagenin 3- and 28-monodesmoside (**1** and **2**), and hederagenin and oleanolic acid 3,28-bisdesmosides (**3**, **5** and **4**).

On alkaline hydrolysis, **5** afforded **1** as its prosapogenin and an oligosaccharide, which afforded glucose and rhamnose on acidic hydrolysis. In addition, analysis of the ^{13}C-NMR spectra of the saponins was carried out, and the saponins **1**, **5**, **3** and **4** were deduced to be identical with kalopanaxsaponins A and B,[4,6] pericarpsaponin P_{J3}[7] and hederasaponin B,[8] respectively. Of them, saponin **5** was shown to be identical with pericarpsaponin P_K[7] (=kalopanaxsaponin B) by direct comparison.

The new saponin named kalopanaxsaponin G (**2**) was shown to be a hederagenin trisaccharide composed of glucose and rhamnose as described above. In the ^{13}C-NMR spectrum of **2**, with respect to those of **1**, **3**, **4** and **5**, the C-28 carboxyl carbon signal appeared at the lower field (−3.9 ppm) than that of hederagenin due to the glycosylation shift although the C-3 and C-23 signals showed no such down-field shift, and the carbon signals due to the sugar moiety were superimposable on those of the trisaccharide moiety in **4** and **5** (Table I). Hence, the structure of **2** was characterized as hederagenin 28-*O*-α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside.

The remaining ten compounds (**6**—**15**) showed hydroxyl and aromatic absorptions in their IR spectra. Of them, seven were characterized as follows, mainly on the basis of coincidence of their physical and chemical properties with those reported: syringin (**7**),[9] protocatechuic acid (**9**), coniferin (**10**),[10] (±)-syringaresinol di-*O*-glucopyranoside (=liriodendrin, **12**),[11] glucosyringic acid (**14**)[9] and chlorogenic acid (**15**). The other four were found to be new compounds, and were named kalopanaxins A (**6**), B (**8**), C (**11**) and D (**13**).

Kalopanaxin A (**6**) showed hydroxyl, aldehyde and aromatic absorptions in its IR spectrum. The ^{13}C-NMR spectrum of **6** exhibited signals due to a methoxyl, an aldehyde, two vinylic and six aromatic carbon atoms, and six signals ascribable to a β-D-glucopyranosyl moiety (Table II), as seen in the spectrum of **12**. The ^{1}H-NMR spectrum showed a methoxyl signal at δ 3.83 and two sets of AMX-type signals, one at δ 7.14 (d, J=8 Hz), 7.29 (dd, J=1, 8 Hz) and 7.40 (d, J=1 Hz), and the other at δ 6.83 (dd, J=8, 16 Hz), 7.66 (d, J=16 Hz) and 9.63 (d, J=8 Hz). Therefore, **6** appeared to be feruloyl (=coniferyl) aldehyde

4-*O*-*β*-D-glucopyranoside. This was proved by chemical conversion of **6** to **10**.

Kalopanaxin B (**8**) showed IR absorption bands due to hydroxyl, ester and aromatic groups. On alkaline treatment in methanol, **8** afforded two products. One was identical with **10**. The other (**16**) showed similar IR absorptions to **8**. The ^{13}C-NMR spectrum of **16** exhibited signals due to an α-L-rhamnopyranosyl moiety, a syringoyl group and a methoxyl carbon (Table II). On acid hydrolysis, **16** gave rhamnose and an aglycone (**17**), which was identical with methyl syringate prepared from **14**.[5b] Accordingly, **16** was identified as methyl syringate 4-*O*-α-L-rhamnopyranoside. On comparison of the ^{13}C-NMR spectra of **8** and **10**, the glucosyl C-6 signal (δ 64.7) of **8** appeared at lower field by 4 ppm than that of **10** (Table II). Consequently, **8** was deduced to be coniferin 6′-*O*-(4-*O*-α-L-rhamnopyranosyl)-syringate.

Kalopanaxin C (**11**) also showed hydroxyl, ester and aromatic absorption bands in its IR spectrum. On alkaline treatment, **11** gave two products. One was identified as **16**. The other (**18**) tested positive in the ferric chloride reaction, and its ^{1}H-NMR spectrum showed the presence of a methoxyl group (δ 3.73) and a 1,2,4-trisubstituted benzene ring (AMX-type signals at δ 6.45, 6.67 and 6.69) in the molecule. The ^{13}C-NMR spectrum of **18** exhibited signals due to a *β*-D-glucopyranosyl moiety (Table II). On acetylation, **18** afforded a penta-acetate (**18a**), which showed no hydroxyl absorption in its IR spectrum. On methylation with diazomethane, **18** gave a dimethyl ether (**18b**), which gave a tetra-acetate (**18c**) under usual acetylation conditions. Accordingly, **18** was concluded to be the glucoside of a monomethylether of 1,2,4-trihydroxybenzene. The location of the substituents on the benzene ring was established by means of the nuclear Overhauser effect (NOE) between aromatic and methoxyl protons of **18a** and **18c**. In the case of **18a**, irradiation of the methoxyl protons at δ 3.79 (3H, s) caused enhancement (18%) of a doublet signal with a *meta*-couping value (J=3 Hz) at δ 6.65 due to the C-3 proton of the benzene ring. For **18c**, irradiation of the methoxyl protons at δ 3.84 (6H, s) caused enhancements (20% each) of the doublet signals at δ 6.61 (J=2 Hz) and δ 6.77 (J=8 Hz) due to the C-3 and C-6 protons, respectively. Hence, **18** was assumed to be 2-methoxy-1,4-hydroquinone 4-*O*-*β*-D-glucopyranoside. This was proved as follows. On acidic hydrolysis, **18b** afforded glucose and an aglycone (**19**), which was identical with 3,4-dimethoxyphenol prepared from vanillin by the Beyer–Villiger oxidation.[12] On comparison of the ^{13}C-NMR spectrum of **11** with that of **18**,

Table II. ^{13}C-NMR Signals of Phenolic Compounds in DMSO-d_6

	7	6	10	13	8	16	14	11	18	15
	SRN	CNA	CNI	CNI	CNI			HDQ	HDQ	FRA
C-1	132.9	128.5	131.1	131.0	131.4			141.5	141.4	125.7
2	104.6	112.0	110.1	109.7	110.2			150.5	150.8	114.4
3	152.8	149.1	149.1	148.9	149.1			102.7	102.6	148.4
4	134.0	148.8	146.0	145.8	145.8			147.8	147.8	145.6
5	152.8	115.6	115.4	115.1	115.3			115.1	115.3	115.8
6	104.6	123.2	119.1	118.9	118.8			107.7	108.1	121.4
7	130.3	153.8	129.0	129.0	129.3			—	—	145.0
8	128.7	126.7	128.5	128.4	128.5			—	—	114.8
9	61.7	195.6	61.7	61.6	61.8			—	—	165.8
OMe	56.6[a]	56.0	55.8	55.6	55.9			55.5	55.6	—
	Glc	Glc	Glc	Glc	Glc			Glc	Glc	QNA
1	102.8	99.9	100.1	98.5	99.9			104.4	101.8	73.6
2	77.3	73.1	73.3	76.9	73.4			73.3	73.4	36.4
3	74.4	76.2	76.9	75.1	76.7			76.4	76.8	70.9
4	70.1	69.7	69.8	70.0	70.5			70.3	70.1	68.2
5	76.7	76.6	77.0	77.2	74.1			73.8	77.4	70.5
6	61.1	60.8	60.8	60.7	64.7			64.7	60.9	37.3
7										174.9
	Api				SGA	SGA	SGA	SGA		
C-1	108.3				125.5	126.3	132.9	125.3		
2	76.1				106.9	107.2	107.7	106.6		
3	79.4				153.2	153.7	152.8	153.0		
4	74.0				138.7	138.8	138.6	138.5		
5	64.5				153.2	153.7	152.8	153.0		
6	—				106.9	107.2	107.7	106.6		
7	—				165.3	167.3	167.6	165.2		
OMe	—				56.5	57.0	56.9	56.2		
OMe	—				56.5	57.0	56.9	56.2		
						53.4				
					Rha	Rha	Glc	Rha		
1					102.3	102.7	102.5	102.2		
2					70.5	71.0	74.6	70.3		
3					70.5	71.0	76.9	70.3		
4					71.9	71.3	70.3	71.7		
5					70.8	71.3	77.6	70.6		
6					18.0	18.3	61.3	17.8		

SRN, syringin; CNA, coniferyl aldehyde; CNI, coniferyl alcohol; SGN, 5-OMe-coniferin; SGA, syringic acid; HDQ, 2-OMe-hydroquinone; FRA, ferulic acid.
a) Doubled.

an acylation shift was observed at the glucosyl C-6 signal (+3.8 ppm), as in the case of **8**. Consequently, the structure of **11** was characterized as 2-methoxyhydroquinone 4-*O*-[6-*O*-(4-*O*-α-L-rhamnopyranosyl)-syringoyl]-β-D-glucopyranoside.

Kalopanaxin D (**13**) showed IR absorption bands due to hydroxyl and aromatic moieties, and ^{13}C-NMR signals due to a coniferyl moiety and two anomeric carbons (Table II), showing **13** to be a glycoside of coniferyl alcohol with two monosaccharide units. Enzymatic hydrolysis of **13** with pectinase furnished glucose and apiose, while mild acid hydrolysis of **13** provided coniferin (**10**). Therefore, **13** was supposed to be an apioside of coniferin. The carbon signal due to the glucopyranosyl C-2 in **13** appeared at lower field by 4 ppm than that of **10**, and the apiosyl group was supposed to be located at C-2 of the glucopyranosyl moiety, as in the case of apiin (=apigenin 7-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside).[13] In the ^{13}C-NMR spectra of **13** and apiin, the signals due to the sugar moieties resembled each other quite closely. Accordingly, **13** was deduced to be coniferyl alcohol 4-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside.

As mentioned above, five saponins and ten phenolic compounds including five new compounds (**2**, **6**, **8**, **11** and **13**) were isolated from the bark of *K. pictus* and were characterized. The new saponin (**2**) is a very rare example of an ester glycoside of oleanolic acid possessing no sugar moiety at the C-3 hydroxyl group, and two of the new phenolic glycosides (**8** and **11**) are remarkable in that they consist of two phenolic glycoside moieties linked together by an ester linkage. Compounds **12** and **7**, obtained in good yield here, were reported to show preventive activity against the stress-induced changes in mice.[14]

It is still necessary to survey the saponin constituents of the root of *K. pictus* and the bark of *K. septemlobus* from the chemotaxonomical point of view.

Experimental

The melting points were determined on a Yanaco micro-melting point apparatus (hot-stage type) and are uncorrected. Optical rotations were measured with a JASCO DIP-140 polarimeter at room temperature (20—25 °C). The IR spectra were taken with a Hitachi EPI-2 spectrometer. The NMR spectra were recorded with a JEOL JNM FX-100 apparatus (100 MHz for ^{1}H-NMR, 25MHz for ^{13}C-NMR) and chemical shifts are given on the δ (ppm) scale with tetramethylsilane as an internal standard. Gas-liquid chromatography (GLC) was run on a Shimadzu GC-6A unit equipped with a flame ionization detector. GLC conditions: column, 5% SE-52 on Chromosorb W (3 mm × 2 m); column temperature, 175 °C; injection temperature, 220 °C; carrier gas, N_2 (1.0 kg/cm^2). Thin-layer chromatography (TLC) was performed on precoated Kieselgel 60 F_{254} plates (Merck) using $CHCl_3$–MeOH–H_2O (70 : 30 : 5, v/v), and detection was achieved by spraying 10% H_2SO_4 followed by heating. Silica gel used for column chromatography was Wako-gel C-200 (Wako Pure Chemical Ind. Co.).

Extraction and Isolation of Saponins from the Bark The fresh bark (without the cork layer, 11.0 kg) of *K. pictus* collected at Narusawa in Yamanashi prefecture, Japan, in June 1982, was chopped after being dried in the air for one week, and extracted with 70% MeOH (15 l × 3) under reflux. The MeOH extract was evaporated to dryness *in vacuo*. The residue (1.96 kg) was suspended in water, and the suspension was extracted with Et_2O and then with BuOH saturated with H_2O. The extracts were concentrated *in vacuo* to give an Et_2O extract (362 g) and a BuOH extract (859 g), respectively. The aqueous layer was subjected to column chromatography on Amberlite XAD-2 eluted first with water and then with MeOH. The MeOH eluate (220 g) and the BuOH extract were combined, followed by evaporation of the solvent, and the residue was subjected to column chromatography on Sephadex LH-20 with MeOH to yield four fractions: fr-**1** (13 g), **2** (595 g) **3** (334 g) and **4** (87 g). Fraction-**2** was repeatedly chromatographed on silica gel columns ($CHCl_3$–MeOH–H_2O (70 : 30 : 5, v/v)) or on Lichroprep RP-8 columns (Merck) (aqueous MeOH) to afford five saponins **1** (50.8 g), **2** (365 mg), **3** (12.9 g), **4** (10.3 g) and **5** (400 g). Fraction-**3** was chromatographed on a silica gel column with $CHCl_3$–MeOH–H_2O (70 : 20 : 2) to give **6** (0.35 g), **7** (1.58 g), **8** (0.71 g), **10** (1.60 g), **11** (1.50 g), **12** (28.9 g), **13** (2.80 g) and **14** (0.17 g). Fraction-**4** was subjected to Toyopearl HW-40 column chromatography with 30% MeOH to give **9** (1.10 g) and **15** (0.54 g).

1: Colorless needles (MeOH), mp 265—268 °C (dec.), $[\alpha]_D$ +18.2° (c=0.3, MeOH) [lit.[6] mp 256—259 °C (dec.), $[\alpha]_D$ +15°]. IR (KBr) cm^{-1}: 3400 (OH), 1700 (COOH). ^{13}C-NMR (Table I): the signals were identical with those reported for clematissaponin CP_{3b} (=kalopanaxsaponin A).[6a] *Anal.* Calcd for $C_{41}H_{66}O_{12}\cdot H_2O$: C, 64.04, H, 8.91. Found: C, 63.46; H, 8.71.

2: Colorless needles (aqueous EtOH), mp 213—215 °C (dec.), $[\alpha]_D$ −2.8° (c=0.3, MeOH). IR (KBr) cm^{-1}: 3300 (OH), 1725 (COOR). ^{13}C-NMR: Table I. *Anal.* Calcd for $C_{48}H_{78}O_{18}\cdot 0.5H_2O$: C, 60.55; H, 8.36. Found: C, 60.78; H, 8.50.

3: A white powder (iso-PrOH), (mp 210—215 °C (dec.)), $[\alpha]_D$ −0.2° (c=0.3, MeOH) [lit.[6d] mp 211—213 °C]. IR (KBr) cm^{-1}: 3400 (OH), 1725 (COOR). ^{13}C-NMR (Table I): the signals due to the sugar moiety at C-28 were super-imposable on those of **5**, and those due to the arabinosyl moiety at C-3 were coincident with those reported for clematissaponin CP_1 (=pericarpsaponin P_{J3}).[6a] *Anal.* Calcd for $C_{53}H_{86}O_{22}\cdot 2H_2O$: C, 57.28; H, 8.16. Found: C, 57.46; H, 8.26.

4: A white powder (iso-PrOH), (mp 210—220 °C (dec.)), $[\alpha]_D$ −29.3° (c=0.8, MeOH). IR (KBr) cm^{-1}: 3400 (OH), 1725 (COOR). ^{13}C-NMR (Table I): the signals resembled those reported for hederasaponin B.[5b] *Anal.* Calcd for $C_{59}H_{96}O_{25}\cdot 2.5H_2O$: C, 56.67; H, 8.14. Found: C, 56.68; H, 8.06.

5: A white powder (iso-PrOH), (mp 226—229 °C (dec.)), $[\alpha]_D$ +20.0° (c=0.7, MeOH) [lit.[6] mp 212—215 °C (dec.), $[\alpha]_D$ +18°]. IR (KBr) cm^{-1}: 3400 (OH), 1725 (COOR). ^{13}C-NMR (Table I): the signals were in accordance with those reported for kalopanaxsaponin B.[5a] Compound **5** was identified as pericarpsaponin P_K (=kalopanaxsaponin B)[7] by direct comparison. *Anal.* Calcd for $C_{59}H_{96}O_{26}\cdot 2.5H_2O$: C, 55.95; H, 8.04. Found: C, 56.04; H, 8.05.

6: Colorless needles (H_2O), mp 209—211 °C, $[\alpha]_D$ −39.3° (c=0.3, pyridine). IR (KBr) cm^{-1}: 3360, 1660, 1590, 1510, 1460. ^{1}H-NMR (DMSO-d_6): 3.83 (3H, s), 6.83 (1H, dd, J=8, 16 Hz), 7.14 (1H, d, J=8 Hz), 7.29 (1H, dd, J=1, 8 Hz), 7.40 (1H, d, J=1 Hz), 7.66 (1H, d, J=16 Hz), 9.63 (1H, d, J=8 Hz). ^{13}C-NMR: Table II. *Anal.* Calcd for $C_{16}H_{20}O_8$: C, 56.47; H, 5.92. Found: C, 56.08; H, 5.85. Reduction of **6** with $NaBH_4$ yielded an alcohol, mp 192—193 °C, which was identified as **10**.

7: Colorless needles (H_2O), mp 192—193 °C, $[\alpha]_D$ −21.4° (c=1.3, MeOH) [lit.[9] mp 190—191 °C, $[\alpha]_D$ −21.8°]. ^{1}H-NMR (DMSO-d_6): 3.77 (3H, s), 4.11 (2H, br t, J=5 Hz), 6.30 (1H, dt, J=5, 17 Hz), 6.50 (1H, d, J=17 Hz), 6.73 (2H, s). ^{13}C-NMR: Table II. *Anal.* Calcd for $C_{17}H_{24}O_9$: C, 54.83; H, 6.50. Found: C, 54.75; H, 6.50. **7** Acetate: Colorlss needles (dil. MeOH), mp 112—114 °C. MS: m/z 582 (M^+, $C_{27}H_{34}O_{14}$). Compound **7** was identified as syringin by direct comparison with an authentic specimen.

8: Colorless needles (H_2O), mp 183 °C, $[\alpha]_D$ −74.9° (c=0.3, pyridine). IR (KBr) cm^{-1}: 3360, 1705, 1590, 1510. ^{1}H-NMR (DMSO-d_6): 1.40 (2H, d, J=6.3 Hz), 3.76 (3H, s), 3.78 (6H, s), 4.09 (2H, t, J=5 Hz), 6.18 (1H, dt, J=16, 5 Hz), 6.43 (1H, d, J=16 Hz), 6.57 (1H, dd, J=1.5, 8 Hz), 6.99 (1H, d, J=8 Hz), 7.03 (1H, d, J=1.5 Hz), 7.24 (2H, s). ^{13}C-NMR: Table II. *Anal.* Calcd for $C_{31}H_{40}O_{16}$: C, 55.69; H, 6.03. Found: C, 55.77; H, 6.04.

9: Colorless needles (H_2O), mp 196—198 °C (dec.). Positive in the $FeCl_3$ reaction: Bluish green. MS: m/z 154 (M^+, $C_7H_6O_4$). IR (KBr) cm^{-1}: 3200, 1670, 1595, 1520, 1460. ^{1}H-NMR (DMSO-d_6): 6.78 (1H, d, J=8 Hz), 7.29 (1H, dd, J=2, 8 Hz), 7.34 (1H, d, J=2 Hz), 9.45 (2H, br s). *Anal.* Calcd for $C_7H_6O_4$: C, 54.55; H, 3.92. Found: C, 54.35; H, 3.86. **9** Acetate: Colorless needles (dil. MeOH), mp 159—160 °C. MS: m/z 238 (M^+, $C_{11}H_{10}O_6$). ^{1}H-NMR ($CDCl_3$): 2.31 (6H, s), 7.41 (1H, d, J=8 Hz), 7.82 (1H, d, J=1.5 Hz), 7.86 (1H, dd, J=8, 1.5 Hz). Compound **9** was identified as protocatechuic acid by direct comparison with a commercially available sample [Wako Pure Chemical Ind. Co.].

10: Colorless needles (H_2O), mp 186—188 °C, $[\alpha]_D$ −63.9° (c=0.3, MeOH) [lit.[10] mp 187 °C, $[\alpha]_D$ −60.0°]. IR (KBr) cm^{-1}: 3300, 1595, 1510, 1460. ^{1}H-NMR (DMSO-d_6): 3.78 (3H, s), 4.09 (2H, br t, J=5 Hz), 4.52 (1H, t, J=5 Hz), 6.25 (1H, dt, J=17, 5 Hz), 6.50 (1H, d, J=17 Hz), 6.89 (1H, dd, J=1.5, 8 Hz), 7.03 (1H, d, J=8 Hz), 7.05 (1H, d, J=1.5 Hz). ^{13}C-NMR: Table II. *Anal.* Calcd for $C_{16}H_{22}O_8$: C, 56.14; H, 6.48. Found: C, 56.14; H, 6.43. **10** Acetate: Colorless needles (dil. MeOH), mp 105—

107 °C [lit.[11b] mp 115 °C]. MS: *m/z* 552 (M^+, $C_{26}H_{32}O_{13}$). Compound **10** was identified as coniferin by direct comparison with an authentic sample.

11: Colorless needles (H_2O), mp 129—130 °C, $[\alpha]_D$ −65.1° (*c*=0.3, pyridine). IR (KBr) cm^{-1}: 3400, 1700, 1690, 1505, 1455. ^{1}H-NMR (DMSO-d_6): 1.08 (3H, d, *J*=6 Hz), 3.66 (3H, s), 3.79 (3H, s), 6.48 (2H, br s), 6.56 (1H, br s), 7.23 (2H, s), 8.49 (1H, s). ^{13}C-NMR: Table II. *Anal.* Calcd for $C_{28}H_{36}O_{16}$: C, 53.50; H, 5.77. Found: C, 53.24; H, 5.80.

12: Colorless needles (H_2O), mp 255 °C. IR (KBr) cm^{-1}: 3340, 1592, 1502, 1420. ^{13}C-NMR: the signals resemble those reported for (+)-syringaresinol di-*O*-β-D-glucopyranoside.[7,11a] *Anal.* Calcd for $C_{34}H_{46}O_{18}\cdot 2H_2O$: C, 52.44: H, 6.44. Found: C, 52.43; H, 6.47. Compound **12** was characterized as a mixture of di-*O*-β-D-glucopyranosides of (+)- and (−)-syringaresinol based on the following evidence. Fractional recrystallization of **12** from water afforded two isomeric compounds, **12a**, mp 248—258 °C, $[\alpha]_D$ +3.2° (*c*=0.3, pyridine) [lit.[11a] mp 258 °C, $[\alpha]_D$ ±0°] and **12b**, mp 210—220 °C, $[\alpha]_D$ −65.2° (*c*=0.2, pyridine) [lit.[11b] mp 245—47 °C, $[\alpha]_D$ −33°]. Enzymatic hydrolysis of **12a** and **12b** with pectinase gave **12a-ag** and **12b-ag** as the individual aglycone, which showed similar spectral data (IR and NMR) to those reported for (+)-syringaresinol.[7,11a] **12a-ag**, colorless needles (MeOH), mp 185—190 °C, $[\alpha]_D$ +50.6° (*c*=0.3, $CHCl_3$) [(+)-syringaresinol,[11a] mp 183.5 °C, $[\alpha]_D$ +44.0°]. **12b-ag**, colorless needles (MeOH), mp 185—190 °C, $[\alpha]_D$ −39.5° (*c*=0.4, $CHCl_3$) [(−)-syringaresinol,[11b] mp 170—172 °C, $[\alpha]_D$ −21.5°].

13: Colorless needles (H_2O), mp 128—130 °C, $[\alpha]_D$ +110.8° (*c*=0.3, pyridine). IR (KBr) cm^{-1}: 3300, 1585, 1510, 1460. ^{1}H-NMR (DMSO-d_6): 3.77 (3H, s), 4.07 (2H, d, *J*=5 Hz), 4.94 (1H, br d, *J*=7 Hz), 5.41 (1H, s), 6.24 (1H, dt, *J*=17, 5 Hz), 6.49 (1H, d, *J*=17 Hz), 6.87 (1H, dd, *J*=2, 8 Hz), 7.00 (1H, d, *J*=8 Hz), 7.05 (1H, d, *J*=2 Hz). ^{13}C-NMR: Table II. *Anal.* Calcd for $C_{21}H_{30}O_{12}\cdot H_2O$: C, 51.22; H, 6.55. Found: C, 51.42; H, 6.08.

14: Colorless needles (MeOH–Et_2O), mp 219—221 °C (dec.) [lit.[9a] mp 212 °C (dec.)], $[\alpha]_D$ −15.2° (*c*=0.3, MeOH). IR (KBr) cm^{-1}: 3400, 1660, 1590, 1500, 1460. ^{1}H-NMR (DMSO-d_6): 3.81 (3H, s), 5.14 (1H, br d, *J*=4 Hz), 7.23 (2H, s), ^{13}C-NMR (DMSO-d_6): Table II. *Anal.* Calcd for $C_{15}H_{20}O_{10}$: C, 50.0; H, 5.60. Found; C, 50.01; H, 5.59. **14** was identical with authentic glucosyringic acid, mp 218—221 °C (dec.), prepared from **7**.[9b]

15: Colorless needles (H_2O), mp 208—209 °C, $[\alpha]_D$ −36.6° (*c*=0.3, MeOH). Positive in the $FeCl_3$ reaction (bluish green). IR (KBr) cm^{-1}: 3280, 1680, 1630, 1590, 1510, 1440. ^{1}H-NMR (DMSO-d_6): 1.89 (4H, m), 3.57 (1H, dd, *J*=3, 7 Hz), 3.93 (1H, m), 5.07 (1H, br d, *J*=7 Hz), 6.14 (1H, d, *J*=16 Hz), 6.76 (1H, d, *J*=8 Hz), 7.00 (1H, dd, *J*=2, 8 Hz), 7.03 (1H, d, *J*=2 Hz), 7.42 (1H, d, *J*=16 Hz), 9.13, 9.57, 12.40 (1H each, s). ^{13}C-NMR: Table II. *Anal.* Calcd for $C_{16}H_{18}O_9$: C, 54.24; H, 5.12. Found: C, 54.13; H, 5.18. Compound **15** was identified as chlorogenic acid by direct comparison with an authentic sample commercially available [Aldrich Chem. Co.].

Acid Hydrolysis of the Saponins (1—5) The saponins were individually subjected to hydrolysis under heating in 2 N HCl in 50% aqueous dioxane on a boiling water bath for 4 h. The reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was worked up in the usual manner, followed by evaporation of the solvent to afford an aglycone. The aglycone (colorless prisms, mp >300 °C; diacetate: colorless needles, mp 172—174 °C) obtained from **1**, **2**, **3** and **5** was identified as hederagenin, and the aglycone (colorless needles, mp 294—295 °C) from **4** was identified as oleanolic acid, by direct comparison with authentic samples. The aqueous layer of the hydrolysate was neutralized with Ag_2CO_3 followed by filtration, and the filtrate was concentrated to dryness *in vacuo*. The residue was examined both on TLC and GLC, and the following sugar components were detected: arabinose (ara), rhamnose (rha) and glucose (glc) from **3**, **4** and **5**; rha and glc from **2**; ara and rha from **1**. TLC, *Rf*: 0.31 (rha), 0.19 (ara), 0.14 (glc). GLC of the tetramethylsilane derivatives, t_R: 3.6, 4.8 (rha), 3.5, 4.0, 4.5 (ara), 11.4, 17.5 (glc).

Alkaline Hydrolysis of 5 Compound **5** (400 mg) was heated at 90 °C with 5% aqueous KOH (20 ml) for 2 h. The reaction mixture was neutralized with 2 N HCl and extracted with BuOH. The BuOH layer was concentrated to dryness *in vacuo*, and the residue was purified by silica gel column chromatography ($CHCl_3$–MeOH–H_2O (80 : 20 : 1, v/v)) to give a prosapogenin (210 mg), colorless needles from MeOH, mp 265—268 °C, $[\alpha]_D$ −10.3° (*c*=0.3, MeOH), which was identified as **1**. Inorganic compounds in the aqueous layer were removed by Sephadex LH-20 column chromatography (MeOH) and the eluate was evaporated to dryness *in vacuo*. The residue was hydrolyzed and the products were examined as described above, and glucose and rhamnose were detected.

Methanolysis of 8 Compound **8** (100 mg) was treated with 0.2% methanolic potassium carbonate under stirring for 30 min at room temperature. After removal of inorganic salts by Sephadex LH-20 chromatography (MeOH), the reaction product was partitioned between EtOAc and water. The product (45 mg), colorless needles (H_2O), mp 186—187 °C, obtained from the aqueous layer was identified as **10**. The product (**16**, 52 mg) from the EtOAc layer: colorless needles (EtOAc), mp 89—91 °C, $[\alpha]_D$ −102.0° (*c*=0.3, pyridine). *Anal.* Calcd for $C_{16}H_{22}O_9$: C, 53.63; H, 6.19. Found: C, 53.44; H, 6.15. IR (KBr) cm^{-1}: 3250, 1705, 1590, 1500, 1460. ^{1}H-NMR (DMSO-d_6+D_2O, 400 MHz): 1.01 (3H, d, *J*=5.2 Hz), 3.29 (1H, t, *J*=9.5 Hz), 3.70 (1H, dd, *J*=3.3, 9.5 Hz), 3.83 (6H, s), 3.86 (3H, s), 3.92 (1H, m), 3.96 (1, dd, *J*=1.5, 3.3 Hz), 3.99 (1H, dd, *J*=5.2, 9.5 Hz), 5.21 (1H, dd, *J*=1.5 Hz), 7.27 (2H, s). ^{13}C-NMR: Table II.

Acid Hydrolysis of 16 Compound **16** (30 mg) was refluxed wih 2 N HCl in 50% aqueous dioxane for 1 h to provide a sugar and an aglycone (**17**, 15 mg), colorless needles, mp 104—105 °C. MS: *m/z* 212 (M^+, $C_{10}H_{12}O_5$). IR (KBr) cm^{-1}: 3400, 1710, 1600, 1500. ^{1}H-NMR (DMSO-d_6): 3.60 (3H, s), 3.95 (6H, s), 5.92 (1H, s), 7.33 (2H, s). Compound **17** was identified as methyl syringate prepared from **14** by methylation with diazomethane, followed by acidic hydrolysis. The sugar was identified as rhamnose, as described above.

Methanolysis of 11 Compound **11** (100 mg) was treated in the same way as described for **8** to afford two products from the EtOAc and the aqueous layers. The one (59 mg), colorless needles (EtOAc), mp 89—91 °C, obtained from the EtOAc layer was identified as **16**. The other (**18**, 38 mg) from the aqueous layer, colorless needles (MeOH), mp 207 °C. IR (KBr) cm^{-1}: 3400, 1610, 1510, 1450. ^{1}H-NMR (DMSO-d_6): 3.73 (3H, s), 6.45 (1H, dd, *J*=2.5, 9 Hz), 6.67 (1H, d, *J*=9 Hz), 6.69 (1H, d, *J*=2.5 Hz), 8.54 (1H, br s). ^{13}C-NMR: Table II.

18 Acetate (**18a**): Colorless needles (MeOH), mp 118—120 °C. MS: *m/z* 512 (M^+, $C_{23}H_{28}O_{13}$). IR (KBr) cm^{-1}: OH (nil), 1720. ^{1}H-NMR ($CDCl_3$): 2.03, 2.05, 2.06, 2.07, 2.29, 3.79 (3H each, s), 6.55 (1H, dd, *J*=3, 9 Hz), 6.59 (1H, d, *J*=9 Hz), 6.65 (1H, d, *J*=3 Hz). Methylation of **18** with diazomethane etherate provided **18b** (37 mg), colorless needles (EtOH), mp 159—161 °C. On acetylation, **18b** provided an acetate (**18c**), colorless needles (EtOH), mp 121—123 °C. MS: *m/z* 484 (M^+, $C_{22}H_{28}O_{12}$). IR (KBr) cm^{-1}: OH (nil), 1720. ^{1}H-NMR ($CDCl_3$): 2.03,2.04, 2.07, 2.08 (3H each, s), 3.84 (6H, s), 6.11 (1H, d, *J*=2 Hz), 6.53 (1H, dd, *J*=2, 8 Hz), 6.77 (1H, d, *J*=8 Hz).

Acid Hydrolysis of 18b Compound **18b** (15 mg) was heated in 2 N HCl in 50% aqueous dioxane (2 ml) at 80 °C for 2 h to give glucose and an aglycone (**19**), colorless syrup. MS: *m/z* 154 (M^+, $C_8H_{10}O_3$). ^{1}H-NMR ($CDCl_3$): 3.82 (6H, s), 6.35 (1H, dd, *J*=3, 9 Hz), 6.48 (1H, d, *J*=3 Hz), 6.74 (1H, d, *J*=9 Hz). ^{13}C-NMR ($CDCl_3$): 55.8 (q), 56.6 (q), 100.7 (d), 105.8 (d), 112.5 (d), 143.2 (s), 149.9 (s), 150.1 (s). Compound **19** was identical with authentic 1,2,4-tri-hydroxybenzene 2,4-dimethylether prepared from vanillin methylether by the Beyer–Villiger oxidation.[12]

Enzymatic Hydrolysis of 13 Compound **13** (5 mg) was incubated with pectinase (0.3 mg) in water (1 ml) at 37 °C for 24 h. The reaction mixture was examined on TLC and GLC, and glucose and apiose were detected. TLC, *Rf*: 0.14 (glc), 0.33 (api). GLC, t_R: 7.3, 11.0 (glc), 1.8, 2.1, 2.4 (api).

Acidic Hydrolysis of 13 Compound **13** (2 mg) was heated in 0.1 N H_2SO_4 at 60 °C for 10 min to give **10** and apiose.

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