Chem. Pharm. Bull. 40(8) 1990—1992 (1992) Vol. 40, No. 8

Triterpenoid Saponins of Aquifoliaceous Plants. V.[1)] Ilexosides XV—XIX from the Barks of *Ilex crenata* THUNB.[2)]

Chieko HATA, Miho KAKUNO, Kazuko YOSHIKAWA,* and Shigenobu ARIHARA

Faculty of Pharmaceutical Sciences, Tokushima-Bunri University, Tokushimashi, Tokushima 770, Japan. *Received January 10, 1992*

Five new saponins, ilexosides XV—XIX, were isolated from the fresh bark of *Ilex crenata*, and their structures were elucidated on the basis of chemical and physicochemical evidence. Ilexosides XV—XVII are 3,28-bisdesmosides of siaresinolic acid, whereas ilexosides XVIII and XIX are those of pomolic acid.

Keywords *Ilex crenata*; Aquifoliaceae; pentacyclic triterpene; ilexoside; pomolic acid; siaresinolic acid; bisdesmoside; saponin

In a previous paper, we reported the isolation and structures of six saponins from the fruit of *Ilex crenata* THUNB.[1)] Continuing with our study of the saponin constituents of this plant we now described the isolation and the structures of five new saponins from the bark of the title plant.

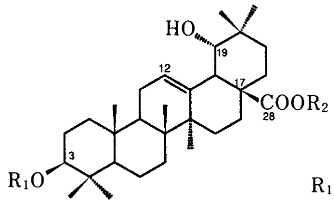
The 70% MeOH extract of the fresh bark (8 kg) of *Ilex crenata* THUNB. was subjected to Amberlite XAD-2 column chromatography to gave a saponin fraction (45 g). Repeated separation of the saponin fraction by reversed-phase octadecyl silica (ODS) and/or ordinary-phase SiO_2 column chromatography furnished five new saponins, ilexosides XV (**1**, 0.54 g), XVI (**2**, 0.14 g), XVII (**3**, 0.04 g), XVIII (**4**, 0.12 g) and XIX (**5**, 0.13 g).

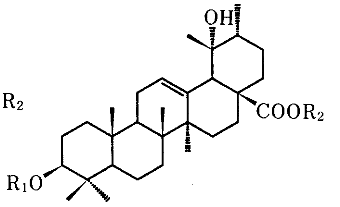
Ilexoside XV (**1**) was obtained as an amorphous powder and had the molecular formula $C_{47}H_{76}O_{18}$ as analyzed by positive fast atom bombardment mass spectrometry (FAB-MS) and carbon counts in the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum. On acid hydrolysis, **1** afforded L-arabinose and D-glucose in the ratio of 1 : 2. The ^{1}H-NMR spectrum of **1** indicated the presence of seven tertiary methyl groups (δ 0.90, 0.99, 0.99, 1.15, 1.17, 1.30, 1.66), one trisubstituted olefinic proton (δ 5.52, br t), one α-arabinosyl unit [H-1: δ 4.76 (d, J=7.5 Hz)], and two β-glucosyl units [H-1: δ 5.37 (d, J=7.5 Hz), 6.36 (d, J=8.0 Hz)]. Compound **1** provided methyl siaresinolate (**6**)[3)] on methanolysis. The electron impact mass spectrum (EI-MS) of **1** acetate showed fragment ion peaks due to a terminal hexosyl cation (*m/z* 331) and a hexosylpentosyl cation (*m/z* 547). The arabinosyl C-3 signal appeared at lower field by +9.5 ppm than that of ziyu-glucoside I (**7**)[4)] because of the glycosylation shift,[5)] demonstrating that a β-glucopyranosyl group is located at the C-3-OH of arabinose. Therefore, **1** was formulated as 3-*O*-β-D-glucopyranosyl (1→3)-α-L-arabinopyranosyl siaresinolic acid 28-*O*-β-D-glucopyranoside.

Ilexoside XVI (**2**), $C_{49}H_{78}O_{19}$ was obtained as an amorphous powder. The FAB-MS of **2** revealed a quasi-molecular ion peak at *m/z* 993 $[M+Na]^+$, 42 mass units more than **1**. The ^{1}H-NMR spectrum of **1** showed the presence of an acetyl group (3H, s at δ 2.29), one α-arabinosyl unit [H-1: δ 4.74 (d, J=8.0 Hz)], and two β-glucosyl units [H-1: δ 5.05 (d, J=7.5 Hz), 6.36 (d, J=8.0 Hz)]. On mild alkaline hydrolysis with 2% KOH, compound **2** afforded **1**. A ^{1}H- and ^{13}C-NMR spectral comparison of **2** with **1**, revealed an acylation shift at the C_2 (position) [+1.40 ppm (H-2), +0.3 ppm (C-2), −2.6 ppm (C-1) and −2.8 ppm (C-3)] of the arabinosyl moiety of **2**. Therefore, in **2**, the *O*-2 of the arabinosyl moiety at C-3 of the agycone should be acetylated. Accordingly, ilexoside XVI was formulated as 3-*O*-[β-D-glucopyranosyl(1→3)]-2-*O*-acetyl-α-L-arabinopyranosyl siaresinolic acid 28-*O*-β-D-glucopyranoside.

Ilexoside XVII (**3**), $C_{53}H_{86}O_{23}$ was obtained as an amorphous powder. The FAB-MS of **3** revealed a quasi-molecular ion peak at *m/z* 1113 $[M+Na]^+$, 162 mass units more than **1**. The ^{1}H- and ^{13}C-NMR spectra of **3** showed similar patterns to those of **1** except for the region of hydroxymethine signals. Compound **3** afforded L-arabinose and D-glucose in the ratio of 1 : 3 on acid hydrolysis. The ^{1}H-NMR spectrum indicated the presence of one α-arabinosyl unit [H-1: δ 4.81 (d, J=7.0 Hz)] and three β-glucopyranosyl units [H-1: δ 5.31 (d, J=7.5 Hz), 5.52 (d, J=8.0 Hz), 6.36 (d, J=8.0 Hz)]. The EI-MS of **3** acetate showed fragment ion peaks due to a terminal hexosyl cation at (*m/z* 331) and a hexosyl(hexosyl)pentosyl cation (*m/z* 835). A ^{13}C-NMR spectral comparison of **3** with **1** revealed a glycosylation shift (+6.8 ppm) at the C-2 signal (from δ 71.9 to 78.7) of the arabinopyranosyl moiety, indicating a β-glucopyranosyl group to be located at C-2 of arabinopyranosyl moiety. Therefore, **3** was formulated as 3-*O*-[β-D-glucopyranosyl(1→2)]-[β-D-glucopyranosyl(1→3)]-α-L-arabinopyranosyl siaresinolic acid 28-*O*-β-D-glucopyranoside.

Ilexoside XVIII (**4**) obtained as an amorphous powder, had the same molcular formula $C_{53}H_{86}O_{23}$ [FAB-MS, *m/z* 1113 $(M+Na)^+$] as **3**. Compound **4** afforded L-arabinose and D-glucose in the ratio of 1 : 3 on acid hydrolysis. The ^{1}H-NMR spectrum of **4** indicated the presence of six tertiary methyl groups (δ 0.88, 1.11, 1.18, 1.25, 1.41, 1.70), one secondary methyl group [δ 1.07 (d, J=6.0 Hz), one trisubstituted olefinic proton (δ 5.56, br t), one α-arabinosyl unit [H-1: δ 4.74 (d, J=7.5 Hz)], and three β-glucosyl units [H-1: δ 5.32 (d, J=7.5 Hz), 5.51 (d, J=7.5 Hz), 6.29 (d, J=7.5 Hz)]. Treatment of **4** with crude cellulase gave pomolic acid (**8**)[6,7)] as the aglycone. The EI-MS of **4** acetate showed the fragment ion peaks due to a terminal hexosyl cation (*m/z* 331) and a hexosyl(hexosyl)pentosyl cation (*m/z* 835). The carbon signals due to the sugar moieties are superimposable on those of **3**, indicating tht the sugar moieties are the same. Therefore, **4** was formulated as 3-*O*-[β-D-glucopyranosyl (1→2)]-[β-D-glucopyranosyl(1→3)]-α-L-arabinopyranosyl pomolic acid 28-*O*-β-D-glucopyranoside.





	R₁	R₂
ilexoside XV (1)	$-\text{Ara}\overset{3}{-}\text{Glc}$	-Glc
XVI (2)	$-\underset{\underset{\text{Ac}}{\vert 2}}{\text{Ara}}\overset{3}{-}\text{Glc}$	-Glc
XVII (3)	$-\underset{\underset{\text{Glc}}{\vert 2}}{\text{Ara}}\overset{3}{-}\text{Glc}$	-Glc
6	-H	$-CH_3$

Ara: α-L-arabinopyranosyl
Glc: β-D-glucopyranosyl
Xyl: β-D-xylopyranosyl

	R₁	R₂
ilexoside XVIII (4)	$-\underset{\underset{\text{Glc}}{\vert 2}}{\text{Ara}}\overset{3}{-}\text{Glc}$	-Glc
XIX (5)	$-\underset{\underset{\text{Ac}}{\vert 2}}{\text{Ara}}\overset{3}{-}\text{Glc}\overset{2}{-}\text{Xyl}$	-Glc
7	-Ara	-Glc
8	-H	-H
9	$-\underset{\underset{\text{Ac}}{\vert 2}}{\text{Ara}}\overset{3}{-}\text{Glc}\overset{2}{-}\text{Xyl}$	-H
10	$-\underset{\underset{\text{Ac}}{\vert 2}}{\text{Ara}}\overset{3}{-}\text{Glc}$	-H

Chart 1

Ilexoside XIX (**5**), $C_{54}H_{86}O_{23}$ was obtained as an amorphous powder and afforded L-arabinose, D-glucose and D-xylose in the ratio of 1 : 2 : 1 on acid hydrolysis. The carbon signals due to the aglycone of **5** are superimposable on those of **4**, indicating that **5** has the same aglycone as **4**. The ^{1}H-NMR spectrum indicated the presence of an acetyl group (3H s, at δ 2.29), one α-arabinosyl unit [H-1: δ 4.66 (d, $J=8.0$ Hz)], two β-glucosyl units [H-1: δ 5.14 (d, $J=6.5$ Hz), 6.26 (d, $J=8.0$ Hz)], and one β-xylosyl unit [H-1: δ 5.06 (d, $J=8.0$ Hz)]. On enzymatic hydrolysis with crude hesperidinase, **5** provided prosapogenins I (**10**) and II (**9**).

Prosapogenin I (**10**), $C_{43}H_{68}O_{14}$, obtained as colorless needles, afforded L-arabinose and D-glucose in the ratio of 1 : 1 on acid hydrolysis. The ^{1}H-NMR spectrum indicated the presence of an acetyl group (3H s, at δ 2.27). The EI-MS of **10** acetate showed the fragment ion peaks due to a terminal hexosyl cation (m/z 331) and a hexosylpentosyl cation (m/z 547). The carbon signals due to the sugar moiety at C-3 of the aglycone are superimposable on those of **2**, indicating that the these compounds have the same sugar moiety at C-3. Accordingly, prosapogenin I is formulated as structure **10**.

Prosapogenin II (**9**), $C_{48}H_{76}O_{18}$ provided L-arabinose, D-glucose and D-xylose in the ratio of 1 : 1 : 1 on acid hydrolysis. The EI-MS of **9** acetate showed fragment ion peaks due to a terminal pentosyl cation (m/z 259), a pentosylhexosyl cation (m/z 547) and pentosylhexosylpentosyl cation (m/z 763). The glucopyranosyl C-2 signal appeared at lower field by +6.9 ppm than that of **7** because of the glycosylation shift, demonstrating that a β-xylopyranosyl group is located at the C-2-OH of glucose. Therefore, prosapogenin II is formulated as structure **9**.

The EI-MS of **5** acetate showed the fragment ion peaks due to a terminal pentosyl cation (m/z 259) and a hexosyl cation (m/z 331), a pentosylhexosyl cation (m/z 547) and a pentosylhexosylpentosyl cation (m/z 763). A ^{13}C-NMR spectral comparison of **5** with **9** revealed a glycosylation shift (−3.4 ppm) at C-28 of **5**, indicating that the glucopyranosyl unit is linked to C_{28}-OH. Hence, **5** was formulated as 3-*O*-β-D-xylospyranosyl(1→2)-β-D-glucopyranosyl(1→3)-2-*O*-acetyl-α-L-arabinopyranosyl pomolic acid 28-*O*-β-D-glucopyranoside.

Experimental

Melting points were measured with a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were taken on a JASCO DIP-140 digital polarimeter. ^{1}H-(400 MHz) and ^{13}C-(100 MHz) NMR spectra were recorded on a JEOL GX-400 spectrometer in pyridine-d_5 solution using tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in δ (ppm) and coupling constants (J values) are given in hertz (Hz). The following abbreviations are used: s = singlet, d = doublet, t = triplet, m = multiplet and br = broad. The EI and FAM-MS were measured with a JEOL JMS-PX303 mass spectrometer. High performance liquid chromatography (HPLC) was carried out with a Waters ALC/GPC 244 instrument. For column chromatography, Silica gel 60 (230—400 mesh, Merck) was used. Thin layer chromatography (TLC), precoated Silica gel 60F-254 (Merck) was used.

Extract and Isolation of Compounds 1—5 Fresh bark (8 kg) of *Ilex crenata* was extracted with 70% MeOH and the MeOH extract, obtained after removal of the solvent under reduced pressure, was passed through an Amberlite XAD-2 column and eluted with MeOH. Crude saponins (45 g) obtained by evaporation of the MeOH eluate were chromatographed with Servachrome XAD-2 (eluted with 30—70% MeOH) to give five fractions, fr. I—V, in order of elution. Fr. V (1.6 g) was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (25 : 8 : 0.5) to give **2** (0.14 g) and **5** (0.13 g). Fraction IV (18.4 g) was separated into six fractions, fr. IVa—IVf, by Sephadex LH-20 (MeOH). Fraction IVc (3.0 g) was chromatographed on silica gel and purified by repeated HPLC (ODS, 35% CH_3CN) to give **1** (0.54 g) and **4** (0.12 g). Fraction III (0.6 g) was chromatographed on silica gel with BuOH–AcOEt–H_2O (4 : 1 : 5, upper layer) to give **3** (0.04 g).

Ilexoside XV (1) Amorphous powder, $[\alpha]_D$ +13.4° ($c=5.0$, MeOH). FAB-MS m/z: 951 [(M+Na)$^+$, $C_{47}H_{76}O_{18}$: 928]. ^{1}H-NMR δ: 0.90, 0.99, 0.99, 1.15, 1.17, 1.30, 1.66 (3H, each, s, *tert*-CH_3 × 7), 3.33 (1H, dd, $J=11.0$, 4.0 Hz, H-3), 3.53 (1H, br s, H-18), 3.59 (1H, d, $J=3.0$ Hz, H-19), 4.76 (1H, d, $J=7.5$ Hz, H-1 of Ara), 5.37 (1H, d, $J=7.5$ Hz, H-1 of Glc), 5.52 (1H, br t, H-12), 6.36 (1H, d, $J=8.0$ Hz, H-1 of esteric Glc). ^{13}C-NMR: Tables I and II.

Ilexoside XVI (2) Amorphous powder, $[\alpha]_D$ +21.8° ($c=1.0$, MeOH). FAB-MS m/z: 993 [(M+Na)$^+$, $C_{49}H_{78}O_{19}$: 970]. ^{1}H-NMR δ: 0.86, 0.90, 0.99, 1.13, 1.13, 1.17, 1.66 (3H, each, s, *tert*-CH_3 × 7), 2.29 (3H, s, acetyl), 3.20 (1H, dd, $J=11.0$, 4.0 Hz, H-3), 3.52 (1H, br s, H-18), 3.60 (1H, d, $J=3.0$ Hz, H-19), 4.74 (1H, d, $J=8.0$ Hz, H-1 of Ara), 5.05 (1H, d, $J=8.0$ Hz, H-1 of Glc), 5.51 (1H, br t, H-12), 5.99 (1H, dd, $J=10.0$, 8.0 Hz, H-2 of Ara), 6.33 (1H, d, $J=8.0$ Hz, H-1 of esteric Glc). ^{13}C-NMR: Tables I and II.

Ilexoside XVII (3) Amorphous powder, $[\alpha]_D$ +56.2° ($c=1.2$, MeOH). FAB-MS m/z: 1113 [M+Na)$^+$, $C_{53}H_{86}O_{23}$: 1090]. ^{1}H-NMR δ: 0.87, 0.99, 1.12, 1.13, 1.17, 1.25, 1.65 (3H, each, s, t-CH_3 × 7), 3.24 (1H, dd, $J=10.0$, 4.0 Hz, H-3), 3.53 (1H, brs, H-18), 3.60 (1H, d, $J=3.0$ Hz, H-19), 4.81 (1H, d, $J=7.0$ Hz, H-1 of Ara), 5.31 (1H, d, $J=7.5$ Hz, H-1 of Glc), 5.52 (1H, d, $J=8.0$ Hz, H-1 of Glc), 5.59 (1H, br t, H-12), 6.36 (1H, d, $J=8.0$ Hz, H-1 of esteric Glc). ^{13}C-NMR: Tables I and II.

Ilexoside XVIII (4) Amorphous powder, $[\alpha]_D$ +3.9° ($c=0.8$, MeOH). FAB-MS m/z: 1113 [(M+Na)$^+$, $C_{53}H_{86}O_{23}$: 1090]. ^{1}H-NMR δ: 0.88, 1.11, 1.18, 1.25, 1.41, 1.70 (3H, each, s, *tert*-CH_3 × 6), 1.07 (3H, d, $J=6.0$ Hz, 30-CH_3), 2.93 (1H, br s, H-18), 3.24 (1H, dd, $J=11.0$, 4.0 Hz, H-3), 4.74 (1H, d, $J=7.5$ Hz, H-1 of Ara), 5.32 (1H, d, $J=7.5$ Hz, H-1 of Glc), 5.51 (1H, d, $J=7.5$ Hz, H-1 of Glc), 5.56 (1H, br t, H-12), 6.29 (1H, d, $J=7.5$ Hz, H-1 of esteric Glc). ^{13}C-NMR: Tables I and II.

Ilexoside XIX (5) Amorphous power, $[\alpha]_D$ +10.2° ($c=5.0$, MeOH). FAB-MS m/z: 1125 [(M+Na)$^+$, $C_{54}H_{86}O_{23}$: 1102]. ^{1}H-NMR δ: 0.87, 0.92, 1.08, 1.17, 1.41, 1.76 (3H, each, s, *tert*-CH_3 × 6), 1.09 (3H, d, $J=6.5$ Hz, 30-CH_3), 2.29 (3H, s, acetyl), 2.92 (1H, br s, H-18), 3.17 (1H, dd, $J=10.5$, 5.5 Hz, H-3), 4.66 (1H, d, $J=8.0$ Hz, H-1 of Ara), 5.06 (1H, d, $J=8.0$ Hz, H-1 of Xyl), 5.14 (1H, d, $J=7.0$ Hz, H-1 of Glc), 5.56 (1H, br t, H-12), 5.96 (1H, dd, $J=10.0$, 8.0 Hz, H-2 of Ara), 6.26 (1H, d, $J=8.0$ Hz, H-1 of esteric Glc). ^{13}C-NMR: Tables I and II.

Methanolysis of 1 A solution of **1** (70 mg) in 1 N HCl–MeOH (2 ml) was heated at 70 °C for 2 h. After work-up as in a usual manner, the crude aglycone (25 mg) was recrystallized from MeOH to give methyl siaresinolate (**6**) (20 mg), mp 181—183 °C (lit$^{3)}$, mp 184—186 °C), $[\alpha]_D$ +46.0° ($c=1.0$, $CHCl_3$) (lit,$^{3)}$ +45.0°). EI-MS m/z: 486 (M)$^+$. ^{1}H-NMR ($CDCl_3$) δ: 0.67, 0.77, 0.90, 0.95, 0.96, 1.00, 1.24 (3H, each, s, *tert*-CH_3 × 7), 3.20 (1H, dd, $J=11.0$, 4.0 Hz, H-3), 3.10 (1H, br s, H-18), 3.33

TABLE I. ^{13}C-NMR Spectral Data for Aglycone Moieties of Compounds **1**—**6**, **8**, **9** and **10** in Pyridine-d_5 (100 MHz)

	1	**2**	**3**	**4**	**5**	**6**	**8**	**9**	**10**
C- 1	38.7	38.6	38.7	39.1	38.9	38.8	38.9	38.6	38.6
C- 2	26.7	26.6	26.6	26.8	26.7	28.1	28.0	27.2	27.2
C- 3	88.8	89.1	89.0	89.5	89.3	78.2	78.2	89.1	89.1
C- 4	39.7	39.4	39.7	39.9	39.4	39.3	39.3	39.4	39.3
C- 5	56.0	55.9	56.0	56.1	56.0	56.0	55.8	55.8	55.8
C- 6	18.8	18.8	18.7	18.9	18.9	18.9	18.9	18.7	18.7
C- 7	33.1	33.1	33.1	33.6	33.7	33.2	33.6	33.5	33.5
C- 8	40.3	40.7	40.2	40.8	40.7	40.0	40.3	40.4	40.4
C- 9	48.4	48.4	48.3	47.9	47.9	47.3	47.7	47.7	47.7
C-10	37.2	37.2	37.2	37.1	37.1	37.5	37.3	37.0	37.0
C-11	24.2	24.3	24.2	24.2	24.3	24.1	24.0	24.0	24.0
C-12	123.4	123.4	123.4	128.6	128.6	123.3	128.1	128.1	128.0
C-13	144.4	144.3	144.3	139.4	139.4	144.3	139.9	140.0	140.0
C-14	42.2	42.2	42.1	42.3	42.3	42.1	42.1	42.1	42.4
C-15	29.0	29.1	29.0	29.4	29.5	29.0	29.2	29.3	29.6
C-16	28.0	28.1	28.0	26.3	26.3	28.1	26.6	26.5	26.5
C-17	46.5	46.6	46.5	48.8	48.9	46.4	48.2	48.3	48.3
C-18	44.7	44.7	44.6	54.6	54.6	44.6	54.5	54.6	54.6
C-19	81.1	81.2	81.0	72.7	72.3	81.0	72.7	72.7	72.7
C-20	35.6	35.6	35.6	42.2	42.3	35.6	42.3	42.2	42.1
C-21	29.1	29.1	29.1	26.8	26.9	29.0	27.0	27.2	29.2
C-22	33.2	33.3	33.2	37.9	38.0	33.2	37.4	38.6	38.5
C-23	28.2	28.1	28.0	28.2	28.3	28.2	28.7	28.0	28.0
C-24	16.9	16.8	16.7	16.9	16.9	16.5	16.7	16.8	16.8
C-25	15.6	15.5	15.5	15.8	15.8	15.5	15.5	15.5	15.4
C-26	17.6	17.6	17.6	17.6	17.6	17.4	17.1	17.2	17.2
C-27	24.7	24.8	24.7	24.8	24.8	24.7	24.6	24.8	24.8
C-28	177.3	177.3	177.3	177.4	177.4	178.7	180.6	180.8	180.7
C-29	28.8	28.9	28.8	27.1	27.2	28.8	26.8	27.2	27.1
C-30	25.0	25.0	24.9	16.9	16.9	24.9	16.4	16.8	16.8
$COOCH_3$						51.7			

TABLE II. ^{13}C-NMR Spectral Data for Sugar Moieties of Compounds **1**—**5**, **7**, **9** and **10** in Pyridine-d_5 (100 MHz)

	1	**2**	**3**	**4**	**5**	**7**	**9**	**10**
Inner Ara								
C-1	107.5	104.9	104.4	104.4	104.0	107.2	103.9	104.8
C-2	71.9	72.2	78.7	78.5	71.7	73.0	71.5	72.2
3-*O*-C-3	84.1	81.3	83.3	83.5	81.8	74.6	81.7	81.3
C-4	69.4	69.6	68.8	68.8	69.5	69.4	69.4	69.5
C-5	67.0	67.1	66.1	66.1	66.9	66.4	66.7	67.1
Terminal Glc (1→3)								
C-1	106.4	106.4	105.5	105.6	105.2		105.1	106.5
C-2	75.7	74.6	75.3	75.3	83.2		83.4	74.6
C-3	78.7	78.4	78.5	78.5	78.5		78.4	78.4
C-4	71.6	71.6	71.5	71.5	71.0		71.1	71.6
C-5	78.4	78.4	78.3	78.2	78.2		78.2	78.4
C-6	62.7	62.8	62.5	62.5	62.5		62.5	62.8
Terminal Glc (1→2) or Xyl (1→2)								
C-1			105.1	105.1	106.4		106.5	
C-2			76.2	76.1	76.0		76.1	
C-3			77.6	77.9	78.2		78.2	
C-4			72.4	72.3	71.1		71.1	
C-5			77.5	77.3	67.4		67.3	
C-6			63.2	63.2				
Glc								
C-1	95.9	95.9	95.9	96.0	96.0	95.5		
C-2	74.2	74.2	74.2	74.0	74.1	74.2		
28-*O*-C-3	79.3	79.3	79.4	79.3	79.3	79.0		
C-4	71.1	71.2	71.1	71.3	71.3	71.6		
C-5	79.0	78.9	79.0	78.8	78.9	79.0		
C-6	62.2	62.4	62.2	62.4	62.5	62.7		
Acetyl		21.6			22.0		21.7	21.5
		170.0			170.0		169.7	169.9

(1H, d, $J=3.0$ Hz, H-19), 3.62 (3H, s, COOMe), 5.46 (1H, br t, H-12). ^{13}C-NMR: Table I.

Alkaline Hydrolysis of 2 Compound **2** (30 mg) was stirred with 2% KOH in 50% EtOH (2 ml) at room temperature for 1 h, then the reaction mixture was neutralized with Amberlite IRC-50 and filtered. The filtrate was chromatographed on a silica gel column with $CHCl_3$–MeOH–H_2O (25 : 8 : 0.5) giving **1** (25 mg).

Enzymatic Hydrolysis of 4 A solution of **4** (50 mg) and crude cellulase (50 mg, Sigma) in EtOH–H_2O (1 : 9) and 0.01 M NaH_2PO_4 buffer (pH 4.0) (4 ml each) was incubated for 72 h at 37 °C. After cooling, the reaction mixture was concentrated to dryness. The residue was chromatographed on a silica gel column with $CHCl_3$–MeOH–H_2O (25 : 4 : 0.5) to give pomolic acid (**8**) (10 ml), colorless needles from MeOH, mp 298—300 °C, $[\alpha]_D$ +55.1° ($c=0.7$, tetrahydrofuran (THF)). IR ν_{max}^{KBr} cm^{-1}: 3400 (br, OH), 1690 (C=O), 1045, 1025. FAB-MS: m/z 473 [(M+H)$^+$, $C_{36}H_{46}O_4$: 472], 495 [(M+Na)$^+$, $C_{36}H_{46}O_4$: 472]. EI-MS m/z 472 (M$^+$), 454 (M$-H_2O$)$^+$, 410 (M$^+-H_2O-CO_2$), 264, 246, 208, 201, 190, 175. ^{1}H-NMR δ: 0.92, 1.04, 1.11, 1.24, 1.47, 1.73 (3H, each, s, *tert*-$CH_3\times6$), 1.13 (3H, d, $J=6.5$ Hz, 30-CH_3), 3.05 (1H, br s, H-18), 3.45 (1H, dd, $J=10.4$, 5.5 Hz, H-3), 5.62 (1H, br t, H-12). ^{13}C-NMR: Table I.

Enzymatic Hydrolysis of 5 A solution of **5** (0.1 g) and crude hesperidinase (0.1 g, Tanabe) in EtOH–H_2O (1 : 9) and 0.01 M NaH_2PO_4 buffer (pH 4.0) (4 ml each) was incubated for 24 h at 37 °C. After cooling, the reaction mixture was concentrated to dryness. The residue was chromatographed on a silica gel column with $CHCl_3$–MeOH–H_2O (25 : 8 : 0.5) to give prosapogenin I (**9**) (20 mg) and II (**10**) (25 mg). **9**, colorless needles from MeOH, mp 285—287 °C, $[\alpha]_D$ +23.7° ($c=3.1$, MeOH). FAB-MS m/z: 963 [(M+Na)$^+$, $C_{48}H_{76}O_{18}$: 940]. ^{1}H-NMR δ: 0.82, 0.90, 1.07, 1.09, 1.44, 1.74 (3H, each, s, *tert*-$CH_3\times6$), 1.13 (3H, d, $J=6.0$ Hz, 30-CH_3), 2.29 (3H, s, acetyl), 3.04 (1H, br s, H-18), 3.18 (1H, dd, $J=10.5$, 5.5 Hz, H-3), 4.65 (1H, d, $J=8.0$ Hz, H-1 of Ara), 5.07 (1H, d, $J=8.0$ Hz, H-1 of Xyl), 5.14 (1H, d, $J=8.0$ Hz, H-1 of Glc), 5.59 (1H, br t, H-12), 5.97 (1H, dd, $J=9.5$, 8.0 Hz, H-2 of Ara). ^{13}C-NMR: Tables I and II. **10**, colorless needles from MeOH, mp 295—297 °C, $[\alpha]_D$ +11.6° ($c=1.0$, MeOH). FAB-MS m/z: 831 [(M+Na)$^+$, $C_{43}H_{68}O_{14}$: 808]. ^{1}H-NMR δ: 0.82, 0.89, 1.08, 1.13, 1.44, 1.75 (3H, each, s, *tert*-$CH_3\times6$), 1.12 (3H, d, $J=6.0$ Hz, 30-CH_3), 2.27 (3H, s, acetyl), 3.06 (1H, br s, H-18), 3.21 (1H, dd, $J=10.5$, 5.5 Hz, H-3), 4.73 (1H, d, $J=7.5$ Hz, H-1 of Ara), 5.18 (1H, d, $J=7.5$ Hz, H-1 of Glc), 5.56 (1H, br t, H-12), 6.03 (1H, dd, $J=9.0$, 8.0 Hz, H-2 of Ara). ^{13}C-NMR: Tables I and II.

Identification of Component Sugars of 1—5, 9 and 10 A solution of each compound (3—4 mg) in 5% H_2SO_4 in 50% EtOH was heated at 100 °C for 3 h. The reaction mixture was diluted with water, neutralized with Amberlite IR-45 and concetrated *in vacuo* to dryness. The mole ratio and enantiomeric character (D or L) of each sugar were determined by using RI detection (Waters 410) and chiral detection (Shodex OR-1), respectively, in HPLC (Shodex RSpak DC-613, 75% CH_3CN, 1 ml/min, 70 °C) by comparison with authentic sugars (10 mM each of L-Ara, D-Glc and D-Xyl). These sugars gave the following peaks: D-(+)-Xyl; 5.75 min, L-(+)-Ara; 6.2 min, D-(+)-Glc; 7.38 min.

Acetylation of 1—5, 9 and 10 Each compound (1—2 mg) was acetylated with Ac_2O–pyridine (each 0.1 ml) at room temperature overnight. Work-up as usual gave a colorless oils in each case, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1760—1750, 1230—1220, 1030—1025.

References

1) Part IV: T. Kakuno, K. Yoshikawa, and S. Arihara, *Phytochemistry*, in press.
2) A part of this work has been orally presented at the 107th Annual Meeting of the Pharmaceutical Society of Japan, Kyoto, April 1987, and the 35th Annual Meeting of the Japanese Society of Pharmacognosy, Niigata, September 1988.
3) R. T. Aplin, W. H. Hui, C. T. Ho, and C. W. Yee, *J. Chem. Soc. (C)*, **1971**, 1067.
4) I. Yoshioka, T. Sugawara, A. Ohsuka, and I. Kitagawa, *Chem. Pharm. Bull.*, **19**, 1700 (1971).
5) R. Kasai, M. Okihara, J. Asakawa, K. Mizutani, and O. Tanaka, *Tetrahedron*, **35**, 1427 (1979).
6) T. Nakanishi, H. Terai, M. Nasu, I. Miura, and K. Yoneda, *Phytochemistry*, **21**, 1373 (1982).
7) C. H. Brieskorn and H. Wunderer, *Chem. Ber.*, **100**, 1252 (1971).