Ardisimamillosides C—F, Four New Triterpenoid Saponins from *Ardisia mamillata*

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Four new triterpenoid saponins, ardisimamilloside C (1), 3-*O*-{α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-3β,16α,28,30-tetrahydroxy-olean-12-en, ardisimamilloside D (2), 3-*O*-{α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-3β,15α,28,30-tetrahydroxy-olean-12-en, ardisimamilloside E (3), 3-*O*-{α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-13β,28-epoxy-3β,16α,29-oleananetriol, and ardisimamilloside F (4), 3-*O*-{α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-3β,16α-dihydroxy-13β,28-epoxy-oleanan-30-oic acid were isolated from the roots of *Ardisia mamillata* HANCE. Structure assignments were established on the basis of highresolution (HR)-FAB-MS, ^{1}H-, ^{13}C-, and two-dimensional (2D)-NMR spectra, and on the chemical evidence.

Key words *Ardisia mamillata*; Myrsinaceae; triterpenoid saponin; ardisimamilloside C, D, E, F

Ardisia mamillata HANCE (Myrsinaceae) is a widely occurring shrub in southern China. Its roots have been traditionally used to treat respiratory tract infections and menstrual disorders.[1] Other plants of this genus have also been used for this purpose, and are well documented in traditional medicine in Southeast Asia.[2] Many saponins have been isolated from *A. crenata*,[3] *A. crispa*,[4] and *A. japonica*.[5] Recently, we isolated two new triterpenoid saponins, ardisimamilloside A, 3-*O*-{α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-3β,16α,28α-trihydroxy-13β,28-epoxy-oleanan-30-al and ardisimamilloside B, 3-*O*-{α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-3β-hydroxy-13β,28-epoxy-oleanan-16-oxo-30-al, from the roots of *A. mamillata*.[6] In this paper, we report the isolation and structural elucidation of four additional new triterpenoid saponins (**1**—**4**), along with three known ones (**5**—**7**) from this species.

Results and Discussion

The roots of *A. mamillata* were extracted with 95% ethanol, and the ethanol extract was partitioned between water and hexane, ethyl acetate and *n*-butanol, respectively. Chromatography of the *n*-butanol extract on silica gel, Lobar RP-18, and Sephadex LH-20, and then after repeated HPLC purification over octadecyl silica (ODS) gel, furnished four new saponins (**1**—**4**) along with three known ones (**5**—**7**).

Compound **1** was obtained as a white powder. The molecular formula $C_{53}H_{88}O_{22}$ was established by analysis of high resolution (HR)-FAB-MS. The ^{13}C-NMR spectral data of **1** revealed 53 carbon signals, 30 of which were assigned to the aglycone part, while 23 were assigned to the carbohydrate moiety. The six sp^3 quaternary carbon signals at δ 15.8, 16.7, 17.2, 27.4, 28.1, and 28.2, and two sp^2 hybrid carbons at δ 122.5 and 144.9 indicated that the aglycone of **1** was an olean-12-en skeleton. ^{13}C-NMR spectral data of the sapogenin part of **1** were similar to those of the known compound, cyclamiretin D.[7] As shown in Table 1, there was a signal at δ 67.2 (CH_2 by distortionless enhancement by polarization fransfer (DEPT)) instead of a resonance due to the 30-CHO group. This signal suggested that the –CHO group was reduced to a hydroxymethyl group. The long-range coupling between H-29 and C-30 in the heteronulear multiple bond spectroscopy (HMBC) spectrum supports the same conclusion. As in cyclamiretin D, the existence of hydroxyl groups at C-3 and C-16 was deduced from the ^{13}C-NMR resonances at δ 89.1 and 73.8, respectively. The configuration of the hydroxyl at C-3 was determined using rotating frame Overhauser enhancement spectroscopy (ROESY). The spatial proximities between H_{ax}-3 (δ 3.16) with H-23 (δ 1.17, 3H), H_{ax}-3 with H-5 (δ 0.72), and H-16 (δ 4.95) with H-28 (δ 3.82) indicated a β-configuration for the 3-OH and an α-configuration for 16-OH. The orientation of 16α-OH was determined by comparing the C-16 (δ 73.8) chemical shift on the ^{13}C-NMR spectrum with that in the literature (16α-OH: *ca.* δ 77.0; 16β-OH: *ca.* δ 64.0).[8] Based on this evidence, the structure of the new sapogenin of **1** was established to be 3β,16α,28,30-tetrahydroxy-olean-12-en.

The ^{1}H- and ^{13}C-NMR data of **1** showed four anomeric signals at δ 4.94 (br s), 5.26 (d, J=7.5 Hz), 5.38 (d, J=7.6 Hz), and 6.42 (br s), and δ 104.4, 103.1, 105.5, and 101.6, respectively. Acid hydrolysis of **1** gave three monosaccharides: arabinose, glucose and rhamnose (1 : 2 : 1), which were analyzed by GC as their alditol acetate derivatives. The absolute configurations of the sugars were shown to be D-glucose, L-arabinose and L-rhamnose according to the method reported by Hara and co-workers.[9] NMR techniques , ^{1}H–^{1}H shift correlation spectroscopy (COSY), homonuclear Hartmann-Hahn spectroscopy (HOHAHA), ^{13}C–^{1}H heteronulear correlated spectroscopy (HETCOR), HMBC, and ROESY, were used to determine the nature of the monosaccharides and sequences of the oligosaccharide chain of **1**. The anomeric configurations and ring sizes of each sugar were obtained following analysis on the H-1 vicinal coupling con-

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Dedicated to the memory of Dr. Kyosuke Tsuda.

Table 1. The ^{13}C-NMR Spectral Data for the Aglycone Moieties of **1**—**4** (125 MHz in Pyridine-d_5)

Carbons	**1**	**2**	**3**	**4**	DEPT
C-1	38.9	39.6	39.3	39.2	CH_2
C-2	26.4	26.4	26.6	26.6	CH_2
C-3	89.1	89.0	89.0	89.1	CH
C-4	39.5	39.4	39.6	39.6	C
C-5	55.8	55.5	55.7	55.6	CH
C-6	18.5	18.8	18.0	18.0	CH_2
C-7	33.3	31.2	34.5	34.5	CH_2
C-8	39.5	41.5	42.5	42.5	C
C-9	47.1	48.1	50.5	50.5	CH
C-10	36.9	37.0	37.0	37.0	C
C-11	23.8	24.0	19.3	19.2	CH_2
C-12	122.5	123.1	32.8 (CH_2)	32.9 (CH_2)	CH
C-13	144.9	146.6	86.6	86.6	C
C-14	42.1	48.2	44.7	44.6	C
C-15	34.7	66.5 (CH)	36.9	36.9	CH_2
C-16	73.8	36.7 (CH_2)	76.4	76.4	CH
C-17	41.3	34.8	44.4	44.3	C
C-18	42.3	43.1	50.1	53.6	CH
C-19	43.1	42.2	36.6	33.3	CH_2
C-20	36.3	36.2	36.8	36.3	C
C-21	28.4	30.1	32.8	32.9	CH_2
C-22	32.0	36.2	31.5	30.0	CH_2
C-23	28.1	28.1	28.0	28.0	CH_3
C-24	16.7	16.7	16.5	16.5	CH_3
C-25	15.8	15.7	16.4	16.5	CH_3
C-26	17.2	17.6	18.6	18.6	CH_3
C-27	27.4	20.9	19.7	19.6	CH_3
C-28	69.6	69.0	78.0	78.1	CH_2
C-29	28.2	28.5	69.8 (CH_2)	29.4	CH_3
C-30	67.2	65.9	32.8 (CH_3)	181.0 (C)	CH_2

stants ($^3J_{HH}$, $^1J_{CH}$), observing their H-1 chemical shifts, and comparing their ^{13}C-NMR spectral data with those of methyl glycosides.[10] From the relatively large H-1 coupling constants (7.5, 7.6 Hz), the anomeric hydroxyl of both glucose moieties should have a β-configuration. In the insensitive nuclei enhanced by polarization transfer (INEPT) spectrum, the CH coupling constant of the signal of C-1 (δ 101.6) was 178 Hz, indicating that the glycosidic bond of rhamnose was linked in the α-configuration. The small H-1 coupling constant of arabinose, which exhibited a broad anomeric proton singlet in its ^{1}H-NMR spectrum, and the correlation between H-1 with H-3 and H-5 in ROESY indicated that the arabinose should also have an α-configuration at its anomeric carbon. Based on these results, the four sugars and their anomeric configurations in **1** were determined to be an α-L-arabinopyranose, two β-D-glucopyranoses and an α-L-rhamnopyranose.

The sequence of the oligosaccharide chain was deduced from ^{13}C shift differences between individual sugar residues and model compounds, and from HMBC and ROESY experiments. The C-1 of arabinose was attached to the 3-OH of aglycone, as indicated by the C-3 chemical shift (δ 89.1) of **1**, the correlation between H-1 (δ 4.94) of arabinose with C-3 of aglycone in HMBC, and between H-1 of arabinose with H-3 (δ 3.16) in ROESY. From the HMBC experiment of **1**, the following correlations were observed: H-1 (δ 5.38) of the terminal glucose with C-2 (δ 80.8) of arabinose; H-1 (δ 5.26) of the inner glucose with C-4 (δ 74.9) of arabinose; and H-1 (δ 6.42) of rhamnose with C-2 (δ 78.1) of the inner glucose. Based on the above findings, the structure of compound **1** was elucidated to be 3-*O*-{α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-3β,16α,28,30-tetrahydroxy-olean-12-en. This is a new triterpenoid saponin, trivially named ardisimamilloside C.

Compound **2** was obtained as a white powder, and had the molecular formula $C_{53}H_{88}O_{22}$ based on the HR-FAB-MS spectrum. The ^{13}C-NMR spectral data of **2** showed 53 carbon signals, 30 of which were assigned to the aglycone part, while 23 were assigned to the carbohydrate moiety. The ^{13}C-NMR spectral data of the sapogenin part of **2** were similar to those of **1**. Comparing the ^{13}C-NMR spectral data of **1** and **2**, there was a lack of any resonance due to C-16 at δ 73.8 in **2**; instead, a signal was observed at δ 66.5 (CH by DEPT). From the HMBC experiment of **2**, the long-range coupling of H-27 (δ 20.9) with δ 66.5 was observed. Therefore, there should be a hydroxyl group at C-15. This assignment was confirmed by the downfield shift at C-13 (+2.7 ppm), C-14 (+6.1 ppm) and at C-15 (+31.8 ppm), and the upfield shift at C-27 (−6.5 ppm) and C-17 (−6.7 ppm). The configuration of the hydroxyl at C-3 and C-15 can be determined using a ROESY experiment. The correlations of H_{ax}-3 (δ 3.17) with H-23 (δ 1.17) and H_{ax}-3 with H-5 (δ 0.69) indicated that the hydroxyl at C-3 should have a β-configuration. The correlation of H-15 (δ 4.74) with H-28 (δ 3.87) indicated that the hydroxyl at C-15 should have an α-configuration. Based on these findings, the structure of the new sapogenin of **2** was established to be 3β,15α,28,30-tetrahydroxy-olean-12-en.

The ^{1}H- and ^{13}C-NMR data of **2** displayed four anomeric signals at δ 4.95 (br s), 5.25 (d, J=7.6 Hz), 5.38 (d, J=7.6 Hz), and 6.41 (br s), and δ 104.5, 103.2, 105.4, and 101.6, respectively. Acid hydrolysis gave three monosaccharides, arabinose, glucose and rhamnose in a ratio of 1 : 2 : 1, which were analyzed by the same method as with **1**. Using the same methods as with **1**, glucose was determined to have a D-configuration, while arabinose and rhamnose were determined to have an L-configuration. In **2**, the same sequence of the oligosaccharide as in **1** was determined using NMR techniques (^{1}H–^{1}H COSY, HOHAHA, HETCOR, HMBC, ROESY). These above findings indicated that the structure of **2** should be 3-*O*-{α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-3β,15α,28,30-tetrahydroxy-olean-12-en. This is a new triterpenoid saponin, trivially named ardisimamilloside D.

Compound **3** was obtained as a white powder. The molecular formula $C_{53}H_{88}O_{22}$ was found to be the same as that of **2** by HR-FAB-MS. The ^{13}C-NMR spectral data of **3** showed 53 carbon signals, 30 of which were assigned to the aglycone part, while 23 were assigned to the carbohydrate moiety. The ^{1}H-NMR spectrum (Table 2) showed signals of six tertiary methyl groups at δ 0.85, 1.03, 1.17, 1.36, 1.56, 1.59, and two pairs of germinal protons at δ 3.45, 3.66 (d, J=7.3 Hz) and δ 3.81, 4.08 (d, J=9.2 Hz) corresponding to two –CH_2–O groups. The ^{13}C-NMR spectral data of **3** were similar to those of the known saponin ardisicrenoside A (**5**).[3] Comparing the ^{1}H-, ^{13}C-NMR spectral data of **3** with those of **5** (Tables 1, 2), only the signals due to C-29 and C-30 were completely different. These findings indicated **3** should be the isomer of ardisicrenoside A. The 13β,28-epoxy bridge and

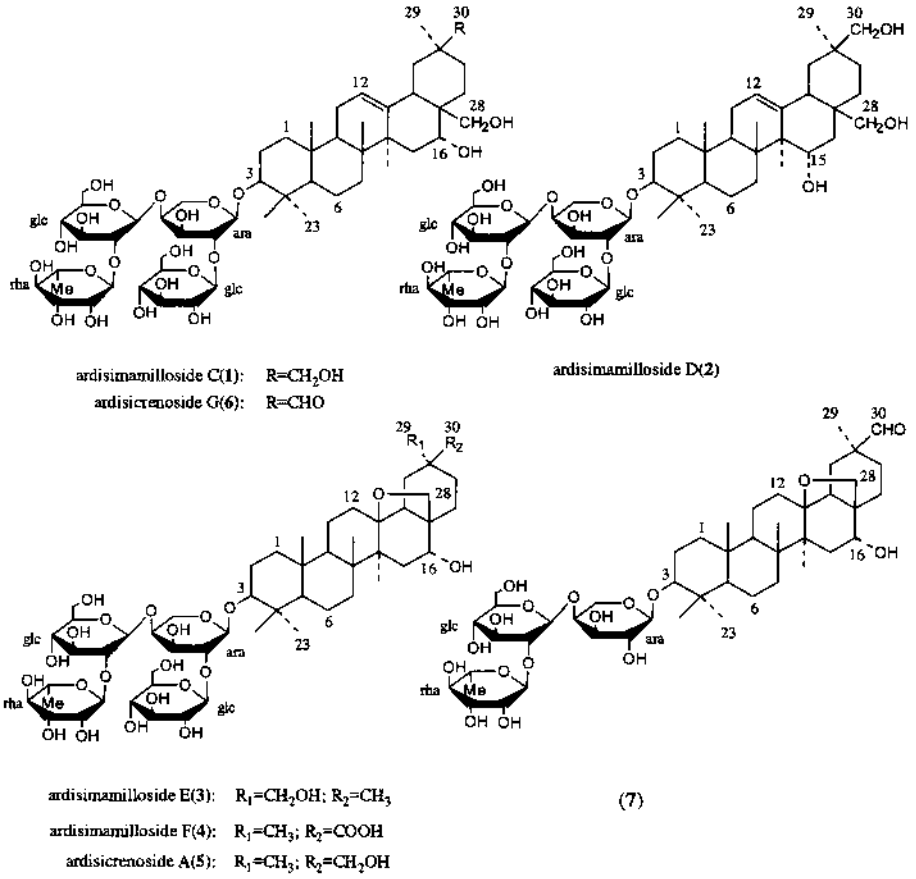

Chart 1

C-16 hydroxyl could be explained by the ^{13}C-NMR resonances at δ 86.6 (C-13), 78.0 (C-28), and 76.4 (C-16), respectively. Furthermore, these assignments could be confirmed through long-range coupling in a HMBC experiment and through spatial interaction in a ROESY experiment. Configurations of hydroxyl at C-3, C-16, and C-20 could be determined using ROESY results. The spatial proximities between H_{ax}-3 (δ 3.14) with H-23 (δ 1.17, 3H), H_{ax}-3 with H-5 (δ 0.66), and H-16 (δ 4.14) with H-28 (δ 3.45, 3.66) indicated a β-configuration for the 3-OH and an α-configuration for 16-OH. A correlation which existed between H-18 and δ 1.56 (3H, s, H-30) indicated that a hydroxymethyl group was located at C-29. From the above evidence, the structure of the new sapogenin of **3** was established to be 13β,28-epoxy-3β,16α,29-oleananetriol.

The ^{1}H- and ^{13}C-NMR data of **3** displayed four anomeric signals at δ 4.95 (br s), 5.25 (d, J=7.6 Hz), 5.38 (d, J=7.6 Hz), and 6.41 (br s), and at δ 104.4, 103.1, 105.4, and 101.6, respectively. Using the same methods as with **1**, the glucose was determined to have a D-configuration, while the arabinose and the rhamnose were determined to have an L-configuration in **3**. The sequence of the oligosaccharide in **3** was established to be the same as in **1** using NMR techniques (^{1}H–^{1}H COSY, HOHAHA, HETCOR, HMBC, ROESY). Therefore, the structure of **3** was determinated to be 3-*O*-{α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-13β,28-epoxy-3β,16α,29-oleananetriol. This is a new triterpenoid saponin, trivially named ardisimamilloside E.

Table 2. The ^{13}C-NMR Spectral Data for the Sugar Units of **1**—**4** (125 MHz in Pyridine-d_5)

Carbons	**1**	**2**	**3**	**4**	DEPT
Arabinose (A)					
A-1	104.4	104.5	104.4	104.4	CH
A-2	80.8	80.8	80.7	80.7	CH
A-3	74.7	74.7	74.7	74.6	CH
A-4	74.9	74.9	74.8	74.8	CH
A-5	62.9	62.8	62.8	62.8	CH_2
Glucose (terminal) (G)					
G-1	105.5	105.4	105.4	105.4	CH
G-2	76.4	76.4	76.4	76.4	CH
G-3	77.3	77.3	77.3	77.1	CH
G-4	71.7	71.7	71.7	71.7	CH
G-5	78.0	78.0	78.0	78.0	CH
G-6	62.9	62.8	62.8	62.8	CH_2
Glucose (inner) (G′)					
G′-1	103.1	103.2	103.1	103.2	CH
G′-2	78.1	78.1	78.1	78.1	CH
G′-3	79.6	79.5	79.5	79.6	CH
G′-4	71.9	71.9	71.8	71.9	CH
G′-5	78.4	78.4	78.4	78.4	CH
G′-6	62.6	62.6	62.6	62.6	CH_2
Rhamnose (R)					
R-1	101.6	101.6	101.6	101.6	CH
R-2	72.4	72.4	72.4	72.4	CH
R-3	72.7	72.7	72.7	72.7	CH
R-4	74.9	74.9	74.9	74.9	CH
R-5	69.4	69.4	69.4	69.4	CH
R-6	19.0	19.0	18.9	18.9	CH_3

Compound **4** was obtained as a white powder. The HR-FAB-MS spectrum of **4** showed quasimolecular ion data at *m/z* 1113.5450, corresponding to the formula $C_{53}H_{86}O_{23}$. The ^{13}C-NMR spectral data of **4** showed 53 carbon signals, 30 of which were assigned to the aglycone part, while 23 were assigned to the carbohydrate moiety. The ^{13}C-NMR spectral data of the sapogenin part of **4** were similar to those of the known triterpene cyclamiretin A.[11] In **4**, there was a lack of any resonance due to the formyl group at C-30 of cyclamiretin A; instead, a signal was observed at δ 181.0 (C by DEPT). This signal suggested that the –CHO group of cyclamiretin A is oxidized to a carboxyl group in **4**. The long-range coupling between H-29 and C-30 in the HMBC experiment supports the same conclusion. As in cyclamiretin A, the 13β,28-epoxy bridge and C-16 hydroxyl could be explained by the ^{13}C-NMR resonances at δ 86.6 (C-13), 78.1 (C-28), and 76.4 (C-16), respectively. Furthermore, these assignments could be confirmed through long-range coupling in the HMBC experiment and through spatial interaction in a ROESY experiment. Configurations of hydroxyl at C-3 and C-16 could be determined using ROESY results. The spatial proximities between H_{ax}-3 (δ 3.15) with H-23 (δ 1.17, 3H), H_{ax}-3 with H-5 (δ 0.65), and H-16 (δ 4.08) with H-28 (δ 3.15, 3.29) indicated a β-configuration for the 3-OH and an α-configuration for 16-OH. From these findings, the structure of the sapogenin of **4** was established to be 3β,16α-dihydroxy-13β,28-epoxy-olean-30-oic acid.

The ^{1}H- and ^{13}C-NMR data of **4** displayed four anomeric signals at δ 4.96 (br s), 5.23 (d, J=7.6 Hz), 5.37 (d, J=7.6 Hz), and 6.41 (br s), and δ 104.4, 103.2, 105.4, and 101.6, respectively. Using the same methods as with **1**, the glucose was determined to have a D-configuration, while the arabinose and the rhamnose were determined to have an L-configuration. In **4**, the same sequence of the oligosaccharide as in **1** was determined using NMR techniques (^{1}H–^{1}H COSY, HOHAHA, HETCOR, HMBC, ROESY). From these findings, the structure of compound **4** was verified to be 3-*O*-{α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-3β,16α-dihydroxy-13β,28-epoxy-olean-30-oic acid. This is a new triterpenoid saponin, trivially named ardisimamilloside F.

Compound **5** was obtained as a white powder. The matrix-assisted laser desorption ionization time of fligh mass spectrum (MALDI-TOF-MS) quasimolecular ion data were at *m/z* 1099 $[M+Na]^+$ and 1115 $[M+K]^+$. On acid hydrolysis, **5** gave rhamnose, glucose, and arabinose (1 : 2 : 1), identified by using the same methods as in **1**. On the basis of its ^{13}C-NMR, COSY, HETCOR, and HMBC experiments, the sequence of its oligosaccharide chain was assigned the same structure as that in the established **1**, and in addition, the attachment of this chain to C-3 of the aglycone was also identified. From the above evidence, **5** was assigned to be 3-*O*-{α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-13β,28-epoxy-3β,16α,30-oleananetriol (ardisicrenoside A), a compound already isolated from *A. crenata*.[3a]

Compound **6** was obtained as a white powder. The MALDI-TOF-MS quasimolecular ion data were at *m/z* 1097 $[M+Na]^+$ and 1113 $[M+K]^+$. On acid hydrolysis, **6** gave rhamnose, glucose, and arabinose (1 : 2 : 1), identified using the same methods as in **1**. On the basis of its ^{13}C-NMR, COSY, HETCOR, and HMBC experiments, the sequence of its oligosaccharide chain was assigned the same structure as that in the established **1**, and in addition, the attachment of this chain to C-3 of the aglycone was also identified. From the above evidence, **6** was assigned to be 3-*O*-{α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-3β,16α,28-olean-12-en-30-al (ardisicrenoside G), a compound already isolated from *A. crenata*.[3b]

Compound **7** was obtained as a white powder. The MALDI-TOF-MS quasimolecular ion data were at *m/z* 935 $[M+Na]^+$ and 951 $[M+K]^+$. On acid hydrolysis, **7** gave rhamnose, glucose, and arabinose (1 : 1 : 1), identified using the same methods as in **1**. On the basis of its ^{13}C-NMR, COSY, HETCOR, and HMBC experiments, the sequence of its oligosaccharide chain was assigned, and in addition, the attachment of this chain to C-3 of the aglycone was also identified. From the above evidence, **7** was assigned to be 3-*O*-{α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-α-L-arabinopyranosyl}cyclamiretin A, a compound already isolated from *Myrsine pellucida*.[12]

Experimental

General Optical rotations were measured using a DIP-1000 digital polarimeter (JASCO corporation). MALDI-TOF-MS and HR-FAB-MS were conducted using Perseptive Voyager RP and a JMS-700K(JEOL) mass spectrometer, respectively. ^{1}H- and ^{13}C-NMR were recorded using a JEOL FT-NMR JNM A-500 spectrometer (^{1}H at 500 MHz, ^{13}C at 125 MHz). Standard pulse sequences were used for the two-dimensional (2D) NMR experiments. Chemical shifts were expressed in δ (ppm) downfield from internal tetramethylsilane (TMS) as an internal standard, and coupling constants (*J*) were reported in Hertz (Hz). TLC was carried out on Silica gel $60F_{254}$, and the spots were visualized by spraying with 10% H_2SO_4 and heating. Silica gel (Silica gel 60—70, 230 mesh, Merck), Lichroprep RP-18 (Lobar, 40—63 μm, Merck) and Sephadex LH-20 were used for column chromatography. Preparative HPLC was performed using an ODS column (PEGASIL ODS, 250×10 mm, Senshu Pak; detector: reflective index and UV 210 nm). GC was run on a Shimadzu GC-14B gas chromatograph (column: Supelco SP-2380 fused silica capillary column; 0.53 mm i.d.×15 m, 0.2 μm film; column temperature: 140 °C→220 °C, 4 °C/min; injection temperature: 250 °C).

Isolation of Saponins The roots of *A. mamillata* Hance were obtained from Sichuan, China in 1996, and the voucher specimens were identified by Prof. Hao Zhang and deposited with the West China University of Medical Sciences. Dried powder (2.5 kg) of the roots of *A. mamillata* was extracted with 95% EtOH (10l×2) under reflux conditions. The EtOH extract (249 g) was partitioned successively between water and hexane, ethyl acetate, and *n*-butanol, respectively. After removing the solvent, the *n*-butanol extract (38.8 g) was dissolved in methanol (40 ml) and the methanol solution was dropped into ether (2.5l) to obtain a precipitate (31.5 g). Ten grams of the ether precipitate were chromatographed on a silica gel column with a solvent system of $CHCl_3$–MeOH–H_2O (7.5 : 2.5 : 0.25). Fractions were combined according their TLC behavior. Fractions 550—670 were chromatographed on a Lichroprep RP-18 column with 30, 50, 70, and 100% MeOH to obtain parts 1—7 (p1—7). Part 1 (412 mg) was chromatographed on a Lichroprep RP-18 column (solvent: CH_3CN : H_2O/1 : 6, 2 : 7) to obtain p1-1—4. P1-1 (23 mg) was chromatographed on a Lichroprep RP-18 column with CH_3CN–H_2O/2 : 8 as an eluate to obtain ardisimamilloside D (**2**, 9.9 mg). P1-2 (41 mg) was purified on a Sephadex LH-20 column with MeOH as an eluate to obtain ardisimamilloside C (**1**, 38.3 mg). P1-3 (44 mg) was isolated on a Lichroprep RP-18 column with CH_3CN : H_2O/2 : 8 as an eluate to obtain ardisimamilloside E (**3**, 29.5 mg). P5 (296 mg) was isolated on a Lichroprep RP-18 column with CH_3CN–H_2O/2 : 8 as an eluate to obtain p5-1—3. Furthermore, p5-2 (118 mg) was isolated on a Lichroprep RP-18 column with CH_3CN–H_2O/2 : 8 as an eluate to obtain ardisicrernoside A (**5**, 19.1 mg). P6 (1 g) was isolated on a Lichroprep RP-18 column with 30—70% MeOH as the gradient eluate to obtain p6-1—7. P6-3 (98 mg) and p6-4 (268 mg) were purified on a Sephadex LH-20 column with MeOH as an eluate to obtain ardisimamilloside F (**4**, 21.3 mg) and ardisicrenoside G (**6**, 248 mg), respec-

tively. P7 (125 mg) was isolated on a silica gel column with $CHCl_3$–MeOH–H_2O/8 : 2 : 0.2 as an eluate to obtain **7** (10 mg).

Ardisimamilloside C (**1**): $[\alpha]_D^{25}$ −28.5° (MeOH; *c*=0.31); HR-FAB-MS (positive): *m/z*: 1099.5696 $[M+Na]^+$ (Calcd for $C_{53}H_{88}O_{22}Na$; 1099.5662). ^{1}H-NMR (500 MHz, pyridine-d_5): δ (ppm) 0.72 (1H, H-5), 0.85 (3H, s, H-25), 0.94 (3H, s, H-24), 1.04 (3H, s, H-26), 1.17 (3H, s, H-23), 1.33(3H, s, H-29), 1.77 (3H, d, *J*=13.2 Hz, H-6 of rhamnose), 1.81 (3H, s, H-27), 2.62 (1H, H-18), 3.16 (1H, dd, *J*=9.8, 4.2 Hz, H-3), 3.82 (2H, s, H-28), 4.14, 4.31 (2H, H-30), 4.94 (1H, br s, H-1 of arabinose), 4.95 (1H, H-16), 5.26 (1H, d, *J*=7.5 Hz, H-1 of inner glucose), 5.38 (1H, d, *J*=7.6 Hz, H-1 of terminal glucose), 6.42 (1H, br s, H-1 of rhamnose). ^{13}C-NMR spectral data are given in Tables 1 and 2.

Ardisimamilloside D (**2**): $[\alpha]_D^{25}$ −21.6° (MeOH; *c*=0.15); HR-FAB-MS (positive): *m/z*: 1099.5671 $[M+Na]^+$ (Calcd for $C_{53}H_{88}O_{22}Na$; 1099.5662). ^{1}H-NMR (500 MHz, pyridine-d_5): δ (ppm) 0.69 (1H, H-5), 0.86 (3H, s, H-25), 1.04 (3H, s, H-24), 1.08 (3H, s, H-26), 1.17 (3H, s, H-23), 1.22 (3H, s, H-29), 1.80 (3H, d, *J*=13.2 Hz, H-6 of rhamnose), 1.64 (3H, s, H-27), 4.74 (1H, H-15), 2.62 (1H, H-18), 3.17 (1H, dd, *J*=10.0, 4.0 Hz, H-3), 3.87 (2H, H-28), 3.89 (2H, H-30), 4.95 (1H, br s, H-1 of arabinose), 5.25 (1H, d, *J*=7.6 Hz, H-1 of inner glucose), 5.38 (1H, d, *J*=7.6 Hz, H-1 of terminal glucose), 6.41 (1H, br s, H-1 of rhamnose). ^{13}C-NMR spectral data are given in Tables 1 and 2.

Ardisimamilloside E (**3**): $[\alpha]_D^{25}$ −25.1° (MeOH; *c*=0.24); HR-FAB-MS (positive): *m/z*: 1099.5679 $[M+Na]^+$ (Calcd for $C_{53}H_{88}O_{22}Na$; 1099.5662). ^{1}H-NMR (500 MHz, pyridine-d_5): δ (ppm) 0.66 (1H, H-5), 0.85 (3H, s, H-25), 1.03 (3H, s, H-24), 1.17 (3H, s, H-23), 1.36 (3H, s, H-26), 1.56 (3H, s, H-30), 1.59 (3H, s, H-27), 1.81 (3H, d, *J*=13.2 Hz, H-6 of rhamnose), 2.62 (1H, H-18), 3.14 (1H, dd, *J*=9.5, 4.0 Hz, H-3), 3.46, 3.66 (2H, d, *J*=7.3 Hz, H-28), 3.81, 4.08 (2H, d, *J*=9.2 Hz, H-29), 4.14 (1H, m, H-16), 4.95 (1H, br s, H-1 of arabinose), 5.25 (1H, d, *J*=7.6 Hz, H-1 of inner glucose), 5.38 (1H, d, *J*=7.6 Hz, H-1 of terminal glucose), 6.41 (1H, br s, H-1 of rhamnose). ^{13}C-NMR spectral data are given in Tables 1 and 2.

Ardisimamilloside F (**4**): $[\alpha]_D^{25}$ −18.6° (MeOH; *c*=0.63); HR-FAB-MS (positive): *m/z*: 1113.5450 $[M+Na]^+$ (Calcd for $C_{53}H_{86}O_{23}Na$; 113.5455). ^{1}H-NMR (500 MHz, pyridine-d_5): δ (ppm) 0.65 (1H, H-5), 0.84 (3H, s, H-25), 1.02 (3H, s, H-24), 1.17 (3H, s, H-23), 1.33 (3H, s, H-26), 1.54 (3H, s, H-29), 1.81 (3H, d, *J*=13.2 Hz, H-6 of rhamnose), 1.60 (3H, s, H-27), 2.62 (1H, H-18), 3.15 (1H, dd, *J*=9.6, 4.2 Hz, H-3), 3.15, 3.29 (2H, d, *J*=7.3 Hz, H-28), 4.08 (1H, H-16), 4.96 (1H, br s, H-1 of arabinose), 5.23 (1H, d, *J*=7.6 Hz, H-1 of inner glucose), 5.37 (1H, d, *J*=7.6 Hz, H-1 of terminal glucose), 6.41 (1H, br s, H-1 of rhamnose). ^{13}C-NMR spectral data are given in Tables 1 and 2.

Ardisicrenoside A (**5**): $[\alpha]_D^{25}$ −12.4° (MeOH; *c*=0.40); MALDI-TOF-MS *m/z*: 1099 $[M+Na]^+$.

Ardisicrenoside F (**6**): $[\alpha]_D^{25}$ −22.9° (MeOH; *c*=0.53); MALDI-TOF-MS *m/z*: 1097 $[M+Na]^+$.

Compound **7**: $[\alpha]_D^{25}$ −27.1° (MeOH; *c*=0.24); MALDI-TOF-MS *m/z*: 935 $[M+Na]^+$.

Compounds **1**—**7** (1 mg each) were hydrolyzed, reduced and acetylated, respectively. The arabitol, glucitol and rhamnitol acetates from compounds **1**—**6** were detected in a ratio of 1 : 2 : 1, and from compound **7** they were detected in a ratio of 1 : 2 : 1 by GC analysis.

The absolute configurations of the sugars were determined according to the method reported by Hara and co-workers[9] using GC. GC conditions: column: 3% ECNSS-M (2 m×0.3 mm); column temp.: 190 °C; injection temp.: 210 °C; retention times (min): L-rhamnose (8.6), L-arabinose (14.4), D-glucose (49.2).

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