Ardisimamillosides G and H, Two New Triterpenoid Saponins from *Ardisia mamillata*

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Two new triterpenoid saponins, ardisimamilloside G (1), 3-*O*-{α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-13β,28-epoxy-16-oxo-oleanan-3β,30-diol and ardisimamilloside H (2), 3-*O*-{α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-α-L-arabinopyranosyl}-3β-hydroxy-13β,28-epoxy-16-oxo-oleanan-30-al, were isolated from the roots of *Ardisia mamillata* HANCE. Structure assignments were established on the basis of spectral data and chemical evidence.

Key words *Ardisia mamillata*; Myrsinaceae; triterpenoid saponin; ardisimamilloside G; ardisimamilloside H

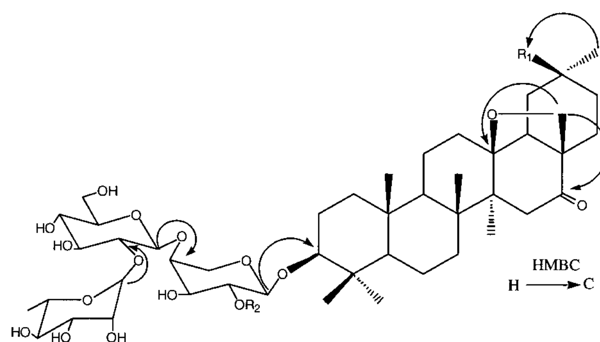
Ardisia mamillata HANCE (Myrsinaceae) is a widely occurring shrub in southern China. Its roots have been traditionally used to treat respiratory tract infections and menstrual disorders.[1] Other plants of this genus have also been used for this purpose, and are well documented in traditional medicine in Southeast Asia.[2] Many saponins have been isolated from *A. crenata*,[3—6] *A. crispa*,[7] and *A. japonica*.[8] Recently, we have isolated six new triterpenoid saponins, ardisimamillosides A—F, from the roots of *A. mamillata*.[9,10] In this paper, we report the isolation and structural elucidation of two additional new triterpenoid saponins (**1**, **2**) 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 1-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 two new saponins (**1**, **2**).

Saponin **1** was obtained as a white powder. The molecular formula $C_{53}H_{86}O_{22}$ of **1** was established by high resolution (HR)-FAB-MS [*m/z* 1097.5505 $(M+Na)^+$]. The ^{13}C-NMR spectral data of **1** revealed showed 53 carbon signals, 30 of which were assigned to the aglycone part, while 23 were assigned to the carbohydrate moiety. The six methyl carbon signals at δ 16.1, 16.4, 18.8, 21.7, 28.0, and 28.8 ppm, and a carbonyl carbon signal at δ 212.9 ppm, indicated that the aglycone of **1** was similar to those of the aglycone of ardisimamilloside B.[9,10] In saponin **1**, there was a lack of the resonance due to the formyl group at C-30 of ardisimamilloside B, instead, a signal was observed at δ 65.0 ppm [CH_2 by distortionless enhancement by polarization transfer (DEPT)]. 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 heteronuclear multiple bond connectivity (HMBC) spectrum supports the same conclusion. As in ardisimamilloside B, the 13β,28-epoxy bridge could be explained by the ^{13}C-NMR resonances at δ 86.3 (C-13) and δ 75.2 ppm (C-28). These assignments could be confirmed through long-range coupling in the HMBC and through spatial interaction in rotating frame Overhauser enhancement spectroscopy (ROESY) experiment. The configuration of the hydroxyl at C-3 was determined using ROESY. The correlation of H_{ax}-3 (δ 3.17 ppm) with H-5 (δ 0.69 ppm) indicated that the hydroxyl at C-3 should have a β-configuration. Based on these findings, the structure of the aglycone of **1** was established to be 13β,28-epoxy-16-oxo-oleanan-3β,30-diol.

Acid hydrolysis of **1** gave three monosaccharides: arabinose, glucose and rhamnose in a ratio of 1 : 2 : 1, which were analyzed by the 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.[11] Both the ^{1}H- and ^{13}C-NMR spectral data of **1** showed four anomeric signals each at δ 4.95 (br s, H-1 of arabinose), 5.25 (d, J=7.6 Hz, H-1 of inner glucose), 5.38 (d, J=7.6 Hz, H-1 of terminal glucose), and 6.41 (br s, H-1 of rhamnose), and δ 104.5, 103.2, 105.5, and 101.6 ppm, respectively. NMR techniques, ^{1}H–^{1}H shift correlation spectroscopy (COSY), ^{13}C–^{1}H heteronuclear 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 constants ($^3J_{HH}$, $^1J_{CH}$), observing their H-1 chemical shifts, and comparing their ^{13}C-NMR spectral data with those of methyl glycosides.[12] 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 nu-



Ardisimamilloside G (**1**): R_1=CH_2OH, R_2=D-Glc

Ardisimamilloside H (**2**): R_1=CHO, R_2=H

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Table 1. The ^{13}C-NMR Spectral Data of Ardisimamillosides G (**1**) and H (**2**) (125 MHz in Pyridine-d_5)

Carbons	1	2	DEPT	Carbons	1	2	DEPT
C- 1	39.1	38.9	CH_2	Arabinose (A)			
C- 2	26.5	26.4	CH_2	A-1	104.5	106.8	CH
C- 3	89.0	88.9	CH	A-2	80.8	72.6	CH
C- 4	39.6	39.4	C	A-3	74.7	73.6	CH
C- 5	55.8	55.8	CH	A-4	74.9	78.3	CH
C- 6	17.9	17.6	CH_2	A-5	62.9	62.4	CH_2
C- 7	33.9	34.0	CH_2	Glucose (terminal) (G)			
C- 8	43.0	42.8	C	G-1	105.5	104.7	CH
C- 9	50.2	49.9	CH	G-2	76.4	78.0	CH
C-10	36.7	36.6	C	G-3	77.4	78.7	CH
C-11	18.9	18.6	CH_2	G-4	71.8	71.6	CH
C-12	31.6	31.9	CH_2	G-5	78.1	78.1	CH
C-13	86.3	86.2	C	G-6	62.6	61.8	CH_2
C-14	45.9	47.7	C	Glucose (inner) (G′)			
C-15	45.8	45.6	CH_2	G′-1	103.2		CH
C-16	212.9	212.8	C	G′-2	78.2		CH
C-17	55.5	55.4	C	G′-3	79.6		CH
C-18	55.8	55.6	CH	G′-4	71.9		CH
C-19	45.3	—	CH_2	G′-5	78.4		CH
C-20	37.1	49.9	C	G′-6	62.6		CH_2
C-21	32.7	29.7	CH_2	Rhamnose (R)			
C-22	31.9	33.6	CH_2	R-1	101.6	101.8	CH
C-23	28.0	27.9	CH_3	R-2	72.4	71.9	CH
C-24	16.4	16.5	CH_3	R-3	72.7	72.1	CH
C-25	16.1	15.9	CH_3	R-4	74.9	73.6	CH
C-26	18.8	18.6	CH_3	R-5	69.5	69.8	CH
C-27	21.7	21.7	CH_3	R-6	19.0	18.5	CH_3
C-28	75.2	74.3	CH_2				
C-29	28.8	23.7	CH_3				
C-30	65.0 (CH_2)	206.3	C				

clear enhanced by polarization transfer (INEPT) spectrum, the CH coupling constant 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 chemical 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 89.0) 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 3.17) 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 5.25) of the inner glucose with C-4 (δ 74.9) of arabinose, and H-1 (δ 6.41) of rhamnose with C-2 (δ 78.0) of inner glucose. Based on these 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}-13β,28-epoxy-16-oxo-oleanan-3β,30-diol. This is a new triterpenoid saponin, trivially named ardisimamilloside G.

Saponin **2** was obtained as a white powder. The molecular formula $C_{47}H_{74}O_{17}$ of **2** was established by HR-FAB-MS [*m/z* 933.4829 $(M+Na)^+$]. The ^{13}C-NMR spectral data of **2** showed 47 carbon signals, 30 of which were assigned to the aglycone part, while 17 were assigned to the carbohydrate moiety. The six methyl carbon signals at δ 15.9, 16.5, 18.6, 21.7, 23.7, and 27.9 ppm, two downfield carbon signals at δ 74.3 ppm and δ 86.2 ppm, and two carbonyl carbon signals at δ 206.3 and δ 212.8 ppm, indicated that the aglycone of **2** was same as that of ardisimamilloside B.[9] Comparing of the ^{1}H-, ^{13}C-NMR spectral data of the aglycone of **2** with those of ardisimamilloside B, the structure of the aglycone of **2** was established to be 3β-hydroxy-13β,28-epoxy-16-oxo-oleanan-30-al.

Acid hydrolysis of **2** gave three monosaccharides, arabinose, glucose and rhamnose in a ratio of 1 : 1 : 1, which were analyzed by the same method as with **1**. The ^{1}H- and ^{13}C-NMR data of **2** displayed three anomeric signals at δ 4.97 (br s, H-1 of arabinose), 5.38 (d, $J=7.6$ Hz, H-1 of glucose), and 6.38 (br s, H-1 of rhamnose), and δ 106.8, 104.7, and 101.8 ppm, respectively. 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 sequence of the oligosaccharide was determined using NMR techniques (^{1}H–^{1}H COSY, HETCOR, HMBC and ROESY) as in **1**. Based on these findings, the structure of **2** should be 3-*O*-{α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-α-L-arabinopyranosyl}-3β-hydroxy-13β,28-epoxy-16-oxo-oleanan-30-al. This is a new triterpenoid saponin, trivially named ardisimamilloside H.

Experimental

General Optical rotations were measured using a DIP-1000 digital polarimeter (JASCO corporation). Matrix-assisted laser desorption/ionization

time-of-flight mass spectra (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). 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: refractive 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 (10 l×2) under reflux conditions. The EtOH extract (249 g) was partitioned successively between water and hexane, ethyl acetate, and 1-butanol, respectively. After removing the solvent, the 1-butanol extract (38.8 g) was dissolved in methanol (40 ml) and the methanol solution was dropped into ether (2.5 l) 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 seven fractions (P1 to P7). P4 (48 mg) was further subjected to a Lichroprep RP-18 column with MeCN–H_2O (10 : 80) to afford ardisimamilloside H (**2**, 4.8 mg). P6 (1 g) was also subjected to a Lichroprep RP-18 column with 30—70% MeOH to give seven fractions (frs. P6-1—7). Fraction P6-5 (276 mg) was purified a Lichroprep RP-18 column with MeCN–H_2O (10 : 80) to afford ardisimamilloside G (**1**, 192 mg).

Ardisimamilloside G (**1**): $[\alpha]_D^{25}$: $-22.6°$ (c=0.83, MeOH); HR-FAB-MS (positive): m/z: 1097.5505 [M+Na]$^+$ (Calcd for $C_{53}H_{86}O_{22}Na$; 1097.5509). ^{1}H-NMR (500 MHz, pyridine-d_5): δ (ppm) 0.69 (1H, br d, J=11.3 Hz, 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), 1.64 (3H, s, H-27), 1.80 (3H, d, J=6.2 Hz, H-6 of rhamnose), 3.17 (1H, dd, J=10.0, 4.0 Hz, H-3), 3.87 (2H, s, H-28), 3.89 (2H, s, 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 Table 1.

Ardisimamilloside H (**2**): $[\alpha]_D^{25}$: $-12.7°$ (c=0.23, MeOH); HR-FAB-MS (positive): m/z: 933.4829 [M+Na]$^+$ (Calcd for $C_{47}H_{74}O_{17}Na$; 933.4824). ^{1}H-NMR (500 MHz, pyridine-d_5): δ (ppm) 0.62 (1H, br d, J=11.3 Hz, H-5), 0.84 (3H, s, H-25), 1.01 (3H, s, H-24), 1.04 (3H, s, H-29), 1.17 (3H, s, H-23), 1.33 (3H, s, H-26), 1.54 (3H, s, H-27), 1.82 (3H, d, J=6.2 Hz, H-6 of rhamnose), 3.16 (1H, dd, J=10.0, 4.0 Hz, H-3), 4.97 (1H, br s, H-1 of arabinose), 5.38 (1H, d, J=7.6 Hz, H-1 of glucose), 6.38 (1H, br s, H-1 of rhamnose), 9.71 (1H, s, H-30). ^{13}C-NMR spectral data are given in Table 1.

Saponins **1** and **2** (1 mg each) were hydrolyzed, reduced and acetylated, respectively. The arabinitol, glucitol and rhamnitol acetates from compounds **1** and **2** were detected in a ratio of 1 : 2 : 1 and 1 : 1 : 1 by GC analysis, respectively.

The absolute configurations of the sugars were determined according to the method reported by Hara and co-workers[11] 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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