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Triterpenoid saponins from *Ardisia mamillata*

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Abstract

Two saponins were isolated from the roots of *Ardisia mamillata* HANCE. Their structures were established on the basis of MALDI-TOFMS, ^{1}H, ^{13}C NMR and 2D NMR (COSY, HOHAHA, HETCOR, HMBC and ROESY) spectra, and on chemical evidence, to be 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.

Keywords: *Ardisia mamillata*; Myrsinaceae; Triterpenoid saponin; Ardisimamilloside A; Ardisimamilloside B

1. Introduction

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 (Jiangsu New Medical College, 1977). Other plants of this genus have also been used for this purpose, and are well documented in traditional medicine in Southeast Asia (Perry and Metzger, 1980). Previous chemical studies have shown that triterpenoid saponins are the main chemical components in this genus. Many saponins have been isolated and characterized from *Ardisia* plants, including six triterpenoid saponins obtained from the roots of *A. crenata* (Jia et al., 1994a, 1994b; Koike et al., 1999; Wang et al., 1992), two utero-contracting saponins obtained from *A. crispa* (Jansakul et al., 1987), three triterpenoid saponins obtained from *A. japonica* (Tomasi et al., 1993) and cyclamiritin A obtained from *A. mamillata* (Li and Xu, 1984), as a hydrolysis product of saponin mixtures. In this paper, we report the isolation and structural elucidation of two new (**1**, **2**) and two known (ardisicrispin B, **3** and cyclaminorin, **4**) triterpenoid saponins, and one known steroid saponin (**5**) from the roots of this species.

2. 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-8 and Sephadex LH-20, followed by repeated HPLC purification over ODS, furnished **1**–**5**.

Compound **1** was obtained as a white powder. The MALDI-TOFMS quasimolecular ion at *m*/*z* 1113 $[M+Na]^{+}$, combined with ^{13}C NMR and DEPT data gave a molecular formula of $C_{53}H_{86}O_{23}$ for **1**. The ^{13}C NMR spectral data of compound **1** revealed 53 carbon

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signals, 30 of which were assigned to the aglycone part, while 23 were assigned to the carbohydrate moiety.

The quasimolecular ion of compound **1** on TOFMS was 16 mass units larger than that of the known saponin **3**. The ^{13}C NMR spectral data of the sapogenin part of **1** were similar to those of **3**, the known oleanane-type triterpene cyclamiritin A. In cyclamiritin A, the 13,28-epoxy bridge and 16α-OH correspond to ^{13}C NMR resonances at δ 86.4 (C-13, C in DEPT), 77.7 (C-28, CH_2) and 76.4 ppm (C-16, CH), respectively. However, in compound **1**, there was a lack of any resonance due to C-28 at δ 77.7 ppm; instead, a signal was observed at δ 99.0 ppm (CH by DEPT). This signal indicated that an –OH group should be at C-28. This assignment was confirmed by the high-field shift at C-17 (−1.5 ppm), and through long-range coupling of H-28 with C-13, C-16 and C-18 in HMBC. The configuration of the hydroxyl at C-3 was determined using ROESY; the correlations of H_{ax}-3 with H-23 (–Me) and H-5 indicated a β-configuration, for the 3-OH. 16-OH showed an α-configuration, as determined by comparing the C-16 (δ 76.4 ppm) chemical shift of the ^{13}C NMR spectrum with that in the literature (16α-OH: ca. δ 77.0 ppm; 16β-OH: ca. δ 64.0 ppm) (Mahata and Kundu, 1994). In the ROESY spectrum, the correlation between 16β-H and 28-H indicated that the configuration of 28-OH should be α. Based on this evidence, the structure of the sapogenin of compound 1 was established to be 3β,16α,28α-trihydroxy-13β,28-epoxy-oleanan-30-al.

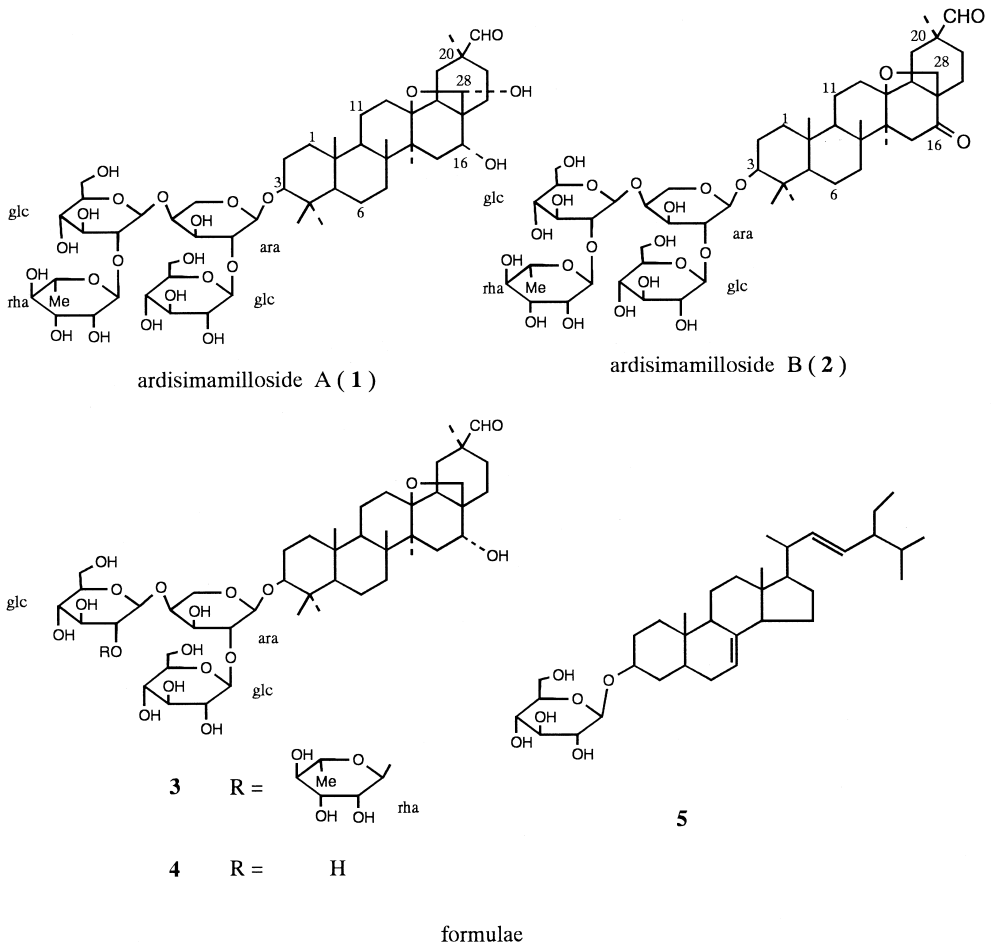
Both the ^1H and ^{13}C NMR spectral data of compound **1** showed four anomeric signals each at δ 4.97 (*br. s*), 5.10 (*d*, $J = 7.5$ Hz), 5.38 (*d*, $J = 7.6$ Hz) and 6.38 (*br. s*), and δ 101.6, 103.2, 104.4 and 105.3 ppm, respectively. Acid hydrolysis of compound **1** gave three monosaccharides — 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 et al. (1987). NMR techniques (^1H–^1H COSY, HOHAHA, HETCOR, HMBC and ROESY) were used to determine the nature of the monosaccharides and sequences of the oligosaccharide chain of compound **1**. The anomeric configurations and ring sizes of each sugar were obtained following analysis of the H-1 vicinal coupling constants ($^3J_{1,2}$ $^1J_{CH}$), observing their H-1 chemical shifts, and comparing their ^{13}C NMR spectral data with those of methyl glycosides (Agrawal, 1992; Agrawal et al., 1985). From the relatively large H-1 coupling constants (7.5 and 7.6 Hz), the anomeric hydroxyls of both glucose moieties should have a β-configuration. In the INEPT experiment, the ^1H–^{13}C coupling constant of C-1 (101.6 ppm) 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 ^1H NMR spectrum, and the correlation between H-1, H-3 and H-5 in the ROESY spectrum, indicated that the arabinose should also have an α-configuration at its anomeric carbon. Based on these results, the four sugars and their anomeric configuration in compound **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 ppm) of compound **1**, by the correlation between H-1 of arabinose and C-3 of aglycone in HMBC and by the ROESY results. Based on the HMBC and ROESY experiments, the following correlations were observed: H-1 (5.38 ppm) of the terminal glucose with C-2 (80.7 ppm) of arabinose; H-1 (5.10 ppm) of the inner glucose with C-4 (74.9 ppm) of arabinose; and H-1 (6.38 ppm) of rhamnose with C-2 (78.1 ppm) of the inner glucose.

Based on the above findings, the structure of compound **1** was elucidated as 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. This is a new triterpenoid saponin, trivially named ardisimamilloside A.

Compound **2** was obtained as a white powder. The MALDI-TOFMS quasimolecular ion at m/z 1095 $[M + Na]^+$, combined with ^{13}C and DEPT NMR data gave a molecular formula of $C_{53}H_{84}O_{22}$ for **2**. The ^{13}C NMR spectral data of compound **2** showed 53 carbon signals, 30 of which were assigned to the aglycone part, while 23 were assigned to the carbohydrate moiety.

The quasimolecular ion of compound **2** on TOFMS was 2 mass units less than the known saponin **3**. The ^{13}C NMR spectral data of the sapogenin part of **2** were similar to those of **3**, which is the known oleanane-type triterpene cyclamiritin A. In **3**, the 13β,28-epoxy bridge and 16α-OH is evident from the ^{13}C NMR resonances at δ 86.4 (C-13, C by DEPT), 77.7 (C-28, CH_2) and 76.4 (C-16, CH) ppm, respectively. However, in compound **2**, no resonance was observed for C-16 at δ 76.4 ppm; instead, a signal was seen at δ 212.6 ppm (C by DEPT), indicating that the –OH at C-16 of compound **3** is oxidized to a carbony1 group. This assignment was confirmed by the low-field shifts at C-17 (+11.2 ppm), C-15 (+8.8 ppm) and C-14 (+3.3 ppm), and the high-field shifts at C-28 (−2.8 ppm). Furthermore, long-range coupling



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of H-28 with C-16 in HMBC also supported the same conclusion. The configuration of the hydroxy1 at C-3 was determined by analysis of ROESY data, wherein the correlation of H_{ax}-3 with H-23 (–Me) and H-5 indicated that the hydroxy1 at C-3 should have a β-configuration. Based on these findings, the structure of the new sapogenin of compound **2** was established to be 3β-hydroxy-13β,28-epoxy-oleanan-16-oxo-30-al.

The ^{1}H and ^{13}C NMR data of compound **2** displayed four anomeric signals each 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 101.6, 103.2, 104.5 and 105.4 ppm, respectively. Acid hydrolysis gave three monosaccharides — arabinose, glucose and rhamnose — in a ratio of 1:2:1. Using the same methods as with compound **1**, glucose was determined to have a D-configuration, while arabinose and rhamnose were determined to have an L-configuration. From the anomeric coupling constants ($^{3}J_{1,2}$, $^{1}J_{CH}$) of the sugar moieties, the two glucoses were determined to have a β-configuration, while arabinose and rhamnose were determined to have an α-configuration. In HMBC experiments, long-range couplings were observed between H-1 (4.95 ppm) of arabinose and C-3 (89.0 ppm) of the aglycone, H-1 (5.38 ppm) of the terminal glucose and C-2 (80.8 ppm) of arabinose, H-1 (5.25 ppm) of inner glucose and C-4 (74.8 ppm) of arabinose, and H-1 (6.41 ppm) of rhamnose and C-2 (78.1 ppm) of the inner glucose. These findings indicated that the structure of compound **2** should be 3-*O*-{α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 4)-[β-D-glucopyranosy1-(1 → 2)]-α-L-arabinopyranosy1]-3β-hydroxy-13β,28-epoxy-oleanan-16-oxo-30-al. This is a new triterpenoid saponin, trivially named ardisimamilloside B.

Compounds **3**, **4** and **5** were identified by comparison of their spectral data (^{1}H, ^{13}C NMR) with literature values (Jia et al., 1994a, 1994b; Jansakul et al., 1987; Lavaud et al., 1994; Calis et al., 1997; Ishii et al., 1980).

3. Experimental

3.1. General

Mps: uncorr. Optical rotations were measured using

a DIP-1000 digital polarimeter (JASCO). MALDI-TOFMS was conducted using a Perseptive Voyager RP mass spectrometer. ^{1}H NMR and ^{13}C NMR spectra 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 2D NMR spectroscopy experiments. Chemical shifts were expressed in δ (ppm) downfield from internal TMS; coupling constants (J) were reported in 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 (60–70, 230 mesh, Merck), Lichroprep RP-8 (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).

3.2. Isolation of saponins

The roots of *A. mamillata* were obtained from Sichuan, China in 1996; the voucher specimens were identified by Prof. Hao Zhang and deposited in 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, ethylacetate, 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 poured into diethy1 ether (2.5 1) following which a precipitate accumulated (31.5 g). Ten grams of the resulting precipitate was subjected to chromatography on a silica gel column, eluted with $CHCl_3$–MeOH–H_2O (7.5:2.5:0.25). Fractions were combined according to their TLC behavior. Frs. 330–430 were recrystallized from MeOH to give **3** (6.2 g). Frs. 441–550 were isolated on Lichroprep RP-8 with 30, 50, 70 and 100% MeOH. The 50% elution was

Table 1
^{13}C NMR spectral data for compounds **1** and **2** (125 MHz in pyridine-d_5)[a]

Aglycone	1	2	DEPT	Sugars	1	2	DEPT
1	39.2	39.0	CH_2	Arabinose (A)			
2	26.5	26.5	CH_2	A-1	104.4	104.5	CH
3	89.0	89.0	CH	A-2	80.7	80.8	CH
4	39.6	39.6	C	A-3	74.7	74.6	CH
5	55.6	55.5	CH	A-4	74.9	74.8	CH
6	18.0	17.8	CH_2	A-5	62.7	62.8	CH_2
7	34.3	33.8	CH_2	Glucose (G) (terminal)			
8	42.6	43.0	C	G-1	105.3	105.4	CH
9	50.3	50.1	CH	G-2	76.3	76.4	CH
10	36.8	36.7	C	G-3	77.3	77.4	CH
11	19.2	18.8	CH_2	G-4	71.7	71.9	CH
12	30.8	31.6	CH_2	G-5	77.9	78.0	CH
13	87.3	86.2	C	G-6	62.6	62.8	CH_2
14	45.0	47.9	C	Glucose (G′) (inner)			
15	36.8	45.7	CH_2	G′-1	103.2	103.2	CH
16	76.4	212.6 (C)	CH	G′-2	78.1	78.1	CH
17	42.6	55.3	C	G′-3	79.5	79.6	CH
18	48.9	55.9	CH	G′-4	71.9	71.8	CH
19	34.3	[b]	CH_2	G′-5	78.3	78.4	CH
20	[b]	50.1	C	G′-6	62.6	62.6	CH_2
21	30.8	29.6	CH_2	Rhamnose (R)			
22	27.8	33.8	CH_2	R-1	101.6	101.6	CH
23	28.1	28.0	Me	R-2	72.4	72.4	CH
24	16.5	16.4	Me	R-3	72.7	72.7	CH
25	16.4	16.1	Me	R-4	74.9	74.8	CH
26	18.6	18.8	Me	R-5	69.5	69.5	CH
27	19.7	21.9	Me	R-6	18.9	18.9	Me
28	99.0 (CH)	74.9	CH_2				
29	24.4	23.9	Me				
30	207.8	206.2	CH				

[a] Assignments based on COSY, HOHAHA, HETCOR and HMBC experiments.
[b] Not observed.

Table 2
^{1}H NMR spectral data for compounds **1** and **2** (500 MHz in pyridine-d_5)[a]

Aglycone	1	2	Sugars	1	2
1	0.78, 1.62	0.76, 1.58	Arabinose (A)		
2	1.88, 2.02	1.80, 2.01	A-1	4.97 (*br. s*)	4.95 (*br. s*)
3	3.16	3.16	A-2	4.58	4.59
4	–	–	A-3	4.27	4.28
5	0.62 (*d*, 11.3)	0.62 (*d*, 11.0)	A-4	4.61	4.60
6	1.42	1.41	A-5	4.32	4.28
7	1.18, 1.52	0.94		4.46	4.48
8	–	–			
9	1.26	1.08	Glucose (G) (terminal)		
10	–	–	G-1	5.38 (*d*, 7.6)	5.38 (*d*, 7.6)
11	1.36, 1.60	1.67	G-2	4.08	4.10
12	2.07, 2.60	1.54	G-3	4.23	4.28
	(*td*, 13.0, 5.5)		G-4	4.27	4.22
13	–	–	G-5	4.33	4.32
14	–	–	G-6	4.28	4.26
15	1.46, 2.20	2.82		4.50	4.49
	(*dd*, 13.5, 5.0)				
16	4.16	–	Glucose (G′) (inner)		
17	–	–	G′-1	5.10 (*d*, 7.5)	5.25 (*d*, 7.6)
18	1.88	1.88	G′-2	4.04	4.00
19	2.17	–	G′-3	4.21	4.20
20	–	–	G′-4	4.13	4.12
21	2.60	1.93	G′-5	3.83	3.82
22	1.85, 2.01	0.96	G′-6	4.25	4.26
23	1.17	1.18		4.50	4.50
24	1.01	1.03			
25	0.84	0.80	Rhamnose (R)		
26	1.33	1.26	R-1	6.38 (*br. s*)	6.41 (*br. s*)
27	1.54	1.10	R-2	4.75	4.75
28	5.14	3.89, 4.32 (*d*, 8.0)	R-3	4.70	4.70
29	1.04	0.91	R-4	4.61	4.60
30	9.71	9.53	R-5	5.04	5.02
			R-6	1.82 (*d*, 6.2)	1.82 (*d*, 6.1)

[a] Assignments based on COSY, HOHAHA, HETCOR, HMBC and ROESY experiments.

purified on HPLC (solvent: MeOH–H_2O 55:45; detector: reflective index) and Sephadex LH-20 with MeOH to give **1** (16.6 mg) and **2** (24.5 mg). Frs. 291–329 were chromatographed on reversed-phase silica gel (Lichroprep RP-8) with 30, 50, 70 and 100% MeOH. The 70% MeOH eluate was further purified on HPLC (solvent : MeOH–H_2O 68:32; detector: UV 210 nm) to give **4** (16 mg). Frs. 9–18 were dissolved in MeOH and purified by silica gel column chromatography with a solvent system of $CHCl_3$–MeOH–H_2O (12.5:2.5:0.25) to give **5** (19.4 mg).

Compound **1**: mp: 235–236°C (dec.); $[\alpha]_D^{25}$: −20.9° (MeOH; *c* 0.23); MALDI-TOFMS *m/z*: 1113 $[M+Na]^+$; Found: C, 55.57; H, 8.10. $C_{53}H_{86}O_{23}\cdot 3H_2O$ requires: C, 55.58; H, 8.12%. ^{1}H and ^{13}C NMR spectral data are given in Tables 1 and 2.

Compound **2**: mp: 261–262°C (dec.); $[\alpha]_D^{25}$: −23.5° (MeOH; *c* 0.24); MALDI-TOFMS: *m/z* 1095 $[M+Na]^+$; Found: C, 57.40; H, 7.98. $C_{53}H_{84}O_{22}\cdot 2H_2O$ requires: C, 57.39; H, 8.00%. ^{1}H and ^{13}C NMR spectral data are given in Tables 1 and 2.

Compounds **1** and **2** of 1 mg each were hydrolyzed, reduced and acetylated. The arabiniol, glucitol and rhamnitol acetates from compounds **1** and **2** were detected by GC analysis in a ratio of 1:2:1.

The absolute configurations of the sugars were determined according to the method reported by Hara et al. (1987) using GC. GC conditions: column: 3% ECNSS-M (2 m × 0.3 mm); column temperature: 190°C; injection temperature: 210°C; RR_t (min): L-rhamnose (8.6), L-arabinose (14.4), D-glucose (49.2).

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