Minor Triterpenoid Saponins from *Ardisia crenata*

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Two minor triterpenoid saponins, ardisicrenoside G [3β-*O*-{α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-16α,28-dihydroxyolean-12-en-30-oic acid] and ardisicrenoside H [3β-*O*-{β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-16α,28-dihydroxyolean-12-en-30-oic acid] were isolated from the roots of *Ardisia crenata*. Structural assignments are based on NMR, MS and chemical reactions.

Key words *Ardisia crenata*; Myrsinaceae; triterpenoid saponin; ardisicrenoside G; ardisicrenoside H

In previous communications, we reported the isolation and structural elucidation of several novel triterpenoid saponins from the roots of *Ardisia crenata*.[1—3] Further investigation led to the isolation of two minor triterpenoid saponins, ardisicrenosides G (**1**) and H (**2**).

The roots of *A. crenata* were defatted with petroleum ether, and then extracted with $CHCl_3$ and MeOH. Chromatography of the MeOH extract on Diaion HP-20, silica gel, octadecyl silica (ODS) gel and then after repeated HPLC purification over ODS, furnished two new saponins, ardisicrenosides G (**1**) and H (**2**).

Ardisicrenoside G (**1**), amorphous powder, $[\alpha]_D$ +27°, possessed the molecular formula $C_{53}H_{86}O_{23}$, as determined by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) MS (at *m/z* 1113 $[M+Na]^+$, 1129 $[M+K]^+$) and from ^{13}C and ^{13}C distortionless enhancement by polarization transfer (DEPT) NMR spectral data. ^1H- and ^{13}C-NMR spectra indicated that **1** had the same aglycone, 3β,16α,28-trihydroxyolean-12-en-30-oic acid (jacquinic acid) and the same oligosaccharide structure at C-3 as that of ardisicrenoside C (**3**) (Tables 1, 2).[2] The component sugars (arabinose : rhamnose : glucose/1 : 1 : 2) were further confirmed by GLC analysis of the TMSi derivatives of the acid hydrolysate. The stereochemistry of each anomeric carbon was determined from comparison of ^1H and ^{13}C data with that of **3**. Thus, ardisicrenoside G was identified to be 3β-*O*-{α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-16α,28-dihydroxyolean-12-en-30-oic acid (**1**).

Ardisicrenoside H (**2**), amorphous powder, $[\alpha]_D$ +64°, showed the molecular formula $C_{52}H_{84}O_{23}$ determined by MALDI-TOF MS (at *m/z* 1099 $[M+Na]^+$, 1115 $[M+K]^+$) and from ^{13}C and DEPT NMR data. The ^1H- and ^{13}C-NMR spectra indicated that **2** possessed the same aglycone as **1** but differed in the sugar part (Tables 1, 2). NMR data indicated that **2** possessed the same sugar arrangement at C-3 as that of ardisicrenoside D (**4**),[2] in which the terminal rhamnose in **1** was replaced by xylose. Thus, ardisicrenoside H was determined to be 3β-*O*-{β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-16α,28-dihydroxyolean-12-en-30-oic acid (**2**).

Table 1. ^{13}C-NMR Data for the Aglycone Moieties (in Pyridine-d_5)

Carbon	1[a]	2	3[b]	Carbon	1	2	3
1	38.9 t	38.8 t	38.7	16	73.8 d	73.8 d	73.3
2	26.4 t	26.4 t	26.1	17	40.5 s	40.5 s	40.1
3	89.1 d	88.9 d	88.9	18	44.2 d	44.2 d	43.0
4	39.5 s	39.6 s	39.2	19	44.8 t	44.8 t	44.3
5	55.7 d	55.9 d	55.5	20	44.6 s	44.6 s	44.4
6	18.5 t	18.4 t	18.2	21	33.9 t	33.9 t	33.3
7	33.2 t	33.2 t	32.9	22	32.6 t	32.6 t	31.5
8	40.1 s	40.1 s	39.8	23	27.4 q	28.1 q	27.9
9	47.1 d	47.1 d	46.8	24	16.6 q	16.9 q	16.4
10	36.9 s	36.9 s	36.6	25	15.5 q	15.7 q	15.4
11	23.8 t	23.8 t	23.5	26	17.0 q	17.1 q	16.7
12	122.5 d	122.8 d	122.8	27	27.4 q	26.4 q	27.1
13	145.1 s	145.1 s	144.3	28	70.9 t	70.9 t	69.8
14	41.9 s	41.9 s	41.5	29	29.4 q	29.4 q	28.3
15	34.9 t	34.9 t	34.6	30	180.5 s	180.4 s	176.9

a) Assignment was based on ^1H–^1H correlation spectroscopy (COSY), heteronuclear correlation spectroscopy (HETCOR), DEPT experiments. *b*) Data taken from reference 2.

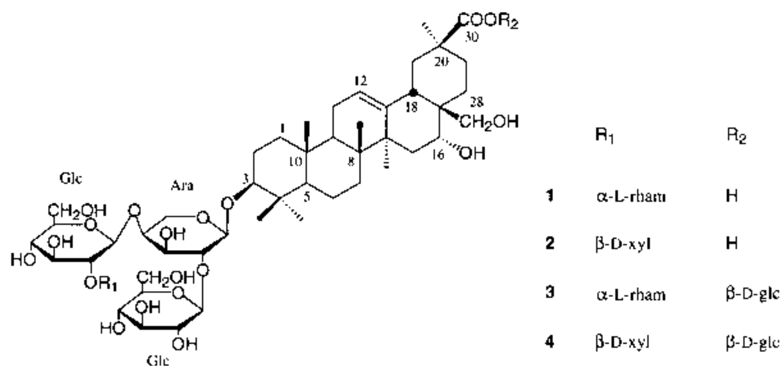

Chart 1

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Table 2. ^{13}C- and ^{1}H-NMR Data for the Sugar Units (in Pyridine-d_5)

Sugar unit	**1**[a]	**2**	DEPT
Arabinose			
A-1	104.5	104.7	CH
A-2	80.8	79.8	CH
A-3	72.5	73.3	CH
A-4	74.8	78.7	CH
A-5	63.6	64.3	CH_2
Glucose (terminal)			
G-1	105.5	104.9	CH
G-2	76.4	76.2	CH
G-3	78.3	78.2	CH
G-4	71.8	71.2	CH
G-5	78.1	78.0	CH
G-6	62.9	63.0	CH_2
Glucose (inner)			
G′-1	103.1	104.3	CH
G′-2	77.3	85.5	CH
G′-3	79.6	77.6	CH
G′-4	71.8	71.9	CH
G′-5	78.4	78.4	CH
G′-6	62.6	62.4	CH_2
Rhamnose			
R-1	101.5		CH
R-2	72.4		CH
R-3	72.7		CH
R-4	74.7		CH
R-5	69.4		CH
R-6	18.9		CH_3
Xylose			
X-1		107.7	CH
X-2		76.1	CH
X-3		77.9	CH
X-4		70.9	CH
X-5		67.5	CH_2

a) Assignment was based on COSY, HETCOR, DEPT experiments.

Experimental

IR spectra were determined using a JASCO 300E FT-IR spectrometer. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. MALDI-TOF MS were conducted using a PerSeptive Biosystems Voyager DE-STR mass spectrometer. ^{1}H-NMR and ^{13}C-NMR were recorded using a JEOL EX-400 (^{1}H at 400 MHz, ^{13}C at 100 MHz) FT-NMR spectrometer. Chemical shifts were expressed in δ (ppm) downfield from tetramethylsilane as an internal standard. Thin layer chromatography (TLC) was carried out on Silica gel 60 F_{254}, and spots were visualized by spraying with 10% H_2SO_4 and heating. Diaion HP-20 (Mitsubishi Chemical), silica gel (Silica gel 60, Merck), and ODS (Chromatorex, 100—200 mesh, Fujisylisia) were used for column chromatography. Preparative HPLC was performed using an ODS column (PEGASIL ODS, Senshu Pak, 10 mm i.d.×250 mm), detector: UV 210 nm. GLC: Shimadzu GC-7A. Column: Silicone OV-17 on Uniport HP (80—100 mesh), 3 mm i.d.×2.1 m; column temperature, 160 °C; carrier gas, N_2, flow rate 30 ml/min.

Extraction and Isolation The roots of *Ardisia crenata* Sims were collected in Jiangxi, China in 1988. The MeOH extract (230 g) from the powdered roots of *A. crenata* (5 kg) was applied to a column of Diaion HP-20 (1.5 kg) and washed with H_2O, 30, 50 ,70, and 100% MeOH to give 50 fractions. The fractions (35—40) containing saponins were combined according to their TLC behaviors. Further purification by silica gel chromatography ($CHCl_3$–MeOH–H_2O/10 : 5 : 1), ODS medium pressure LC (Lichroprep RP-18, MeOH–H_2O/7 : 3) and finally by HPLC (MeOH–H_2O/3 : 2) afforded the minor saponins, ardisicrenosides G (**1**, 8 mg) and H (**2**, 5 mg).

Ardisicrenoside G (**1**): White powder, $[\alpha]_D^{22}$ +27° (MeOH; c=0.26). IR ν_{max}^{KBr}: 3413, 2931, 1680, 1073 cm^{-1}. MALDI-TOF MS m/z 1113 [M+Na]$^+$, 1129 [M+K]$^+$. ^{1}H-NMR (400 MHz, pyridine-d_5): δ 0.85, 0.96, 1.04, 1.18, 1.53, 1.88 (each 3H, s, CH_3 of C-25, C-26, C-24, C-23, C-29, C-27), 1.81 (3H, d, J=5.9 Hz, CH_3-6 of rha), 2.65 (1H, dd, J=16.8, 1.5 Hz, H-18), 3.16 (1H, dd, J=11.0, 4.3 Hz, H-3), 3.57, 3.84 (each 1H, d, J=10.4 Hz, H_2-28), 4.84 (1H, br s, H-16), 4.94 (1H, d, J=4.7 Hz, H-1 of ara), 5.27 (1H, d, J=7.7 Hz, H-1 of inner glc), 5.39 (1H, d, J=7.7 Hz, H-1 of terminal glc), 5.62 (1H, br t, H-12), 6.43 (1H, br s, H-1 of rha). ^{13}C-NMR data: Tables 1 and 2.

Ardisicrenoside H (**2**): White powder, $[\alpha]_D^{22}$ +64° (MeOH; c=0.16). IR ν_{max}^{KBr}: 3414, 2929, 1680, 1074 cm^{-1}. MALDI-TOF MS m/z 1099 [M+Na]$^+$, 1115 [M+K]$^+$. ^{1}H-NMR (400 MHz, pyridine-d_5): δ 0.86, 0.96, 1.10, 1.23, 1.53, 1.90 (each 3H, s, CH_3 of C-25, C-26, C-24, C-23, C-29, C-27), 3.17 (1H, dd, J=11.5, 4.0 Hz, H-3), 4.85 (1H, br s, H-16), δ 4.79 (1H, d, J=5.9 Hz, H-1 of ara), 4.92 (1H, d, J=6.5 Hz, H-1 of xyl), 5.02 (1H, d, J=7.7 Hz, H-1 of inner glc), 5.51 (1H, d, J=7.5 Hz, H-1 of terminal glc), 5.63 (1H, br t, H-12). ^{13}C-NMR data: Tables 1 and 2.

Acid Hydrolysis Compound **1** (2 mg) was heated in 1 ml 1 M HCl (dioxane–H_2O, 1 : 1) at 80 °C for 1 h on a water bath. Dioxane was removed and the solution extracted with EtOAc (1 ml×3 times). The monosaccharide portion was neutralized by passing through an ion-exchanged resin (Amberlite MB-3) column, concentrated and then treated with 1-(trimethylsilyl)imidazole at room temperature for 2 h. After excess reagent was decomposed with water, the reaction product was extracted with hexane (1 ml×3 times). The TMSi derivatives of the monosaccharides were identified as those of arabinose, glucose, and rhamnose in a ratio of 1 : 2 : 1 by GLC. By the same method, the sugars in **2** were identified to be arabinose, glucose, and xylose (1 : 2 : 1).

References

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