Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2021, **19**(1): 63-69
doi: 10.1016/S1875-5364(21)60007-9
Chinese Journal of Natural Medicines

•Research article•

Triterpenoid saponins and phenylpropanoid glycoside from the roots of *Ardisia crenata* and their cytotoxic activities

SONG Ning-Ning[1], YANG Lei-Min[1], ZHANG Min-Jie[1], AN Ren-Feng[1], LIU Wei[2], HUANG Xue-Feng[1*]

[1] *School of Chinese Pharmacy, China Pharmaceutical University, Nanjing 210009, China;*
[2] *Department of Pharmacology, Nanjing Medical University, Nanjing 210029, China*

Available online 20 Jan., 2021

[ABSTRACT] Two new triterpenoid saponins, ardisicrenoside R and S (**1** and **2**), and one new phenylpropanoid glycoside, ardicrephenin (**3**), along with five known compounds (**4**−**8**), were isolated from roots of *Ardisia crenata*. Their structures were elucidated on the basis of NMR spectroscopic data and chemical methods. Compounds **2**−**7** were evaluated for their cytotoxic activities against A549, MCF-7, HepG2 and MDA-MB-231 cell lines by MTT assay. Ardicrenin (**6**) showed significant cytotoxicity, with IC_{50} values of 1.17 ± 0.01, 1.19 ± 0.06, 3.52 ± 0.23, and 16.61 ± 1.02 μmol·L^{-1}, respectively.

[KEY WORDS] *Ardisia crenata*; Triterpenoid saponins; Phenylpropanoid glycoside; Cytotoxicity
[CLC Number] R284.1 **[Document code]** A **[Article ID]** 2095-6975(2021)01-0063-07

Introduction

Ardisia crenata (*A. crenata*), belonging to the Myrsinaceae family, is a widely occurring evergreen shrub in Southeast Asia and North America. Its root, a well-known traditional Chinese medicine called "Zhu Sha Gen", has been used to treat respiratory tract infections and menstrual disorders in traditional Chinese medicine [1]. Previous phytochemical and pharmacological investigations on the roots of *A. crenata* exhibited a number of bioactivities, such as antitumor [2-4], antibacterial [5], antiviral [6], and anti-oxidative effects [7]. Triterpenoid saponins are the main bioactive components. Earlier investigations on this plant resulted in the isolation of more than 20 triterpenoid saponins [8-11]. This study deals with the isolation and identification of three new compounds named as ardisicrenoside R (**1**) ardisicrenoside S (**2**), and ardicrephenin (**3**), together with five known triterpenoid saponins (**4**−**8**) (Fig. 1). Their structures were elucidated using spectroscopic methods and were evaluated for cytotoxic activities against A549, MCF-7, HepG2, and MDA-MB-231 cells.

Results and Discussion

Compound **1** was obtained as a white powder with $[\alpha]_D^{20}$ + 2.22 (*c* 0.05, MeOH). The molecular formula of **1** was determined as $C_{52}H_{86}O_{22}$ by HR-ESI-MS (*m/z* 1080.5940, $[M + NH_4]^+$, Calcd. for $C_{52}H_{90}NO_{22}$, 1080.5949) and indicated ten degrees of unsaturation. The ^{1}H NMR data (Table 1) revealed the presence of six methyl groups at δ_H 0.82 (s, H-25), 0.90 (s, H-26), 1.05 (s, H-24), 1.17 (s, H-23), 1.34 (s, H-29), and 1.84 (s, H-27). An olefinic proton signal at δ_H 5.43 (brs, H-12), connected [according to the HMBC spectrum (Fig. 2)] to carbons at δ_C 16.1 (C-25), 17.5 (C-26), 17.2 (C-24), 28.5 (C-23), 28.5 (C-29), 27.7 (C-27) and 122.8 (C-12) indicative of **1** was an olean-12-en skeleton [12]. ^{13}C NMR spectral data of the sapogenin part of **1** were similar to those of the known compound cyclamiretin D [13]. As shown in Table 1, there were signal at δ_C 67.9 (C-30), 69.9 (C-28) suggested that was a hydroxymethyl group. NOESY correlations between H-3 (δ 3.18) with H-23 (δ 1.17), H-3 (δ 3.18) with H-5 (δ 0.74), and H-16 (δ 4.63) with H-28 (δ 3.95) indicated *β*-configuration for the 3-OH and *α*-configuration for 16-OH. Thus, the aglycone was identified as 3*β*,16*α*,28,30-tetrahydroxy-olean-12-ene.

After acid hydrolysis of **1** with 2 mol·L^{-1} HCl afforded monosaccharides, which were identified by gas chromatographic (GC) analysis [14] of their trimethylsilyl L-cysteine derivatives as L-arabinose, D-glucose and D-xylose in a ratio of 1 : 2 : 1. The ^{1}H NMR data of **1** showed four anomeric signals at δ_H 4.80 (d, *J* = 5.8 Hz, Ara-H-1′), 5.53 (d, *J* = 7.6 Hz, Glc-H-1″), 5.02 (d, *J* = 7.8 Hz, Glc-H-1‴) and 4.92 (d, *J* = 6.5 Hz, Xyl-H-1″). The arabinose was connected to C-3 of

[Received on] 10-Apr.-2020
[*Corresponding author] E-mail: hxf@cpu.edu.cn
These authors have no conflict of interest to declare.

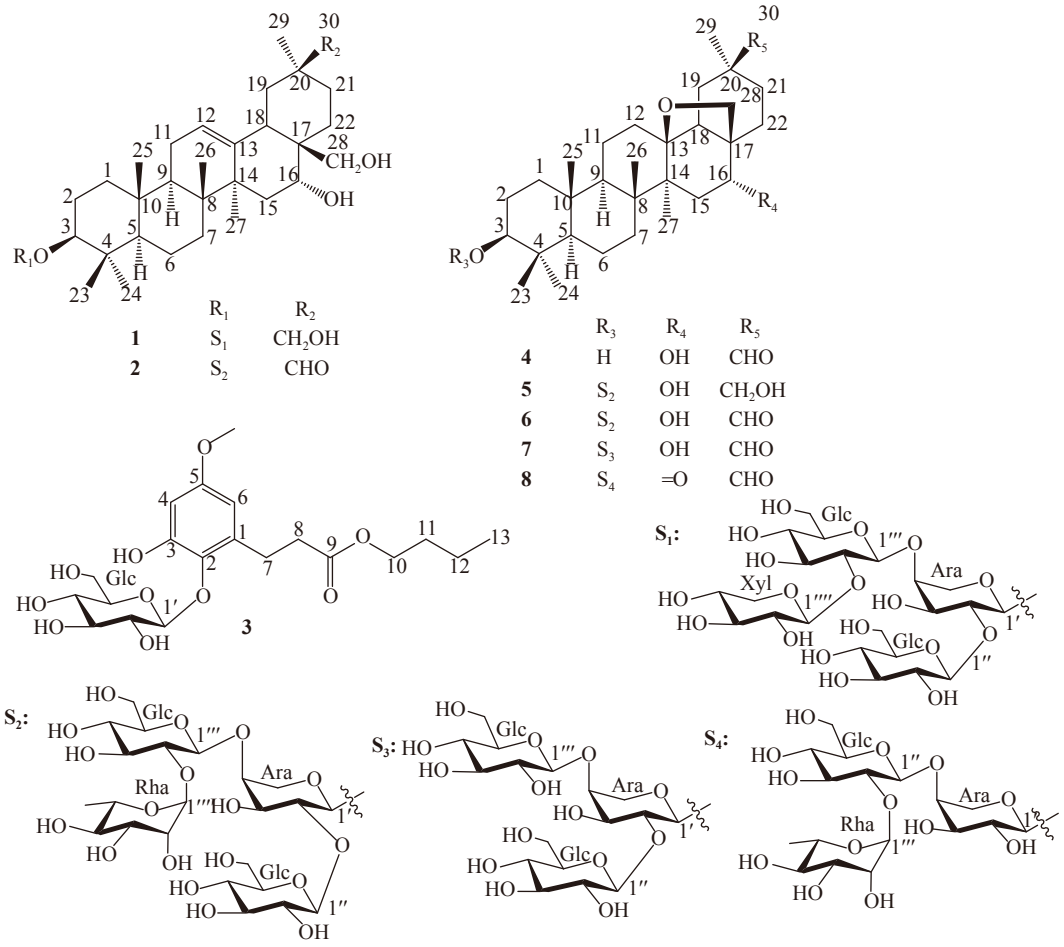

Fig. 1 Structures of compounds 1–8

the aglycone, which was deduced from the HMBC (Fig. 2) correlation to be between Ara-H-1′ (δ_H 4.80) and C-3 (δ_C 89.3). The arabinose unit was determined to be the *α*-anomer on the basis of the $^3J_{H1, H2}$ value (5.8 Hz) and the correlations between H-1′ and H-3′ and between H-1′ and H-5′ in the NOESY experiment. In the HMBC spectrum of **1**, the long-range correlations between H-1″ (δ_H 5.53) and C-2′ (δ_C 80.1), between H-1″ (δ_H 4.92) and C-2‴ (δ_C 85.8), and between H-1‴ (δ_H 5.02) and C-4′ (δ_C 72.2). On the base of the above data, the structure of compound **1** was identified as 3*β*-*O*-{*β*-D-xylopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→4)-[*β*-D-glucopyranosyl-(1→2)]-*α*-L-arabinopyranosyl}-3*β*,16*α*,28,30-tetrahydroxy-olean-12-en. This is a new triterpenoid saponin, trivially named ardisicrenoside R.

Compound **2** was obtained as a white amorphous powder with $[\alpha]_D^{20}$ −5.2 (*c* 0.08, MeOH), whose molecular formula was determined to be $C_{53}H_{86}O_{22}$ by HR-ESI-MS (*m/z* 1097.5495, $[M + Na]^+$, Calcd. for $C_{53}H_{86}O_{22}Na$, 1097.5503). The proton and carbon signals in the ^1H and ^{13}C NMR spectra (Table 1) of **2** were similar to those of **1** except for there was a lack of any resonance due to C-30 at δ 67.9 in **2**, instead, a signal was observed at δ 208.2. From the HMBC (Fig. 2) experiment of **2**, the long-range coupling of H-30 (δ_H 9.80) with C-19 (δ_C 47.9), C-20 (δ_C 31.2) and C-21 (δ_C 42.4) was observed. This signal suggested that the 30-hydroxymethyl group was reduced to a -CHO group. The HMBC correlations from H-28 (δ_H 3.72) to C-17 (δ_C 41.0), C-18 (δ_C 44.0) and C-22 (δ_C 31.8) established the hydroxymethyl group was linked to C-17. According ROESY experiment, the spatial proximities between H-3 (δ_H 3.16) with H-23 (δ_H 1.18), H-3 (δ_H 3.16) with H-5 (δ_H 0.71), and H-16 (δ_H 4.70) with H-28 (δ_H 3.48) indicated a *β*-configuration for the 3-OH and an *α*-configuration for 16-OH. By comparing the NMR data of the sugar chains attached to C-3 of **2** with those of **1**, the suggested that the sugar moiety in **2** is 3-*O*-{*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→4)-[*β*-D-glucopyranosyl-(1→2)]-*α*-L-arabinopyranoside. This was confirmed by the HMBC correlations between H-3 (δ_H 3.16) and C-1′ (δ_C 104.9), H-1″ (δ_H 5.40) and C-2′ (δ_C 81.3), H-1‴ (δ_H 5.29) and C-4′ (δ_C 75.4), H-1″ (δ_H 6.45) and C-2‴ (δ_C 78.6). The structure of **2** was shown to be 3*β*-*O*-{*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→4)-[*β*-D-glucopyranosyl-(1→2)]-*α*-L-arabinopyranosyl}-3*β*,16*α*,28-trihydroxy-olean-12-ene-30-al. This is a new triterpenoid saponin, trivially named ardisicrenoside S.

Compound **3** was obtained as a yellow amorphous

Table 1 ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) date for compounds 1–3 in pyridine-d_5 (J in Hz)

No.	1		2		3	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	39.2	0.90 (2H, m)	37.3	0.91 (2H, m)	136.7	-
2	26.8	1.98 (1H, m); 1.61 (1H, m)	26.9	2.19 (1H, m); 1.38 (1H, m)	137.3	-
3	89.3	3.18 (1H, dd, 11.6, 4.3)	89.6	3.16 (1H, m)	153.1	-
4	39.9	-	40.0	-	100.2	6.74 (1H, d, 2.6)
5	56.2	0.74 (1H, d, 11.7)	56.2	0.71 (1H, d, 11.7)	155.6	-
6	18.8	1.61 (1H, m); 1.37 (1H, m)	18.9	1.63 (1H, m); 1.40 (1H, m)	108.6	6.80 (1H, d, 2.6)
7	33.6	1.74 (1H, m); 1.28 (1H, m)	33.6	2.20 (1H, m); 1.29 (1H, m)	26.5	3.45 (2H, m)
8	40.4	-	40.5	-	35.1	2.90 (1H, m); 3.00 (1H, m)
9	47.5	1.90 (1H, brs)	47.5	1.71 (1H, m)	173.4	-
10	37.2	-	37.3	-	63.9	4.00 (2H, t, 6.7)
11	24.1	2.32 (1H, m); 1.92 (1H, m)	24.3	1.38 (1H, m); 1.85 (1H, m)	30.6	1.42 (2H, m)
12	122.8	5.43 (1H, brs)	123.5	5.43 (1H, m)	19.1	1.17 (2H, m)
13	145.3	-	145	-	13.5	0.73 (3H, t, 7.4)
14	42.4	-	42.3	-		
15	35.1	2.24 (1H, m); 1.50 (1H, m)	35.3	2.20 (1H, m); 1.63 (1H, m)		
16	74.1	4.63 (1H, brs)	74.4	4.70 (1H, brs)		
17	41.7	-	41.0	-		
18	42.6	2.61 (1H, dd, 14.0, 2.4)	44.0	2.34 (1H, dd, 14.0, 4.1)		
19	43.5	2.73 (1H, t, 14.0); 1.37 (1H, m)	47.9	2.81 (1H, t, 13.4); 1.38 (1H, m)		
20	36.7	-	31.2	-		
21	28.7	1.47 (1H, m)	42.4	2.81 (1H, m)		
22	32.3	2.38 (1H, m); 1.37 (1H, m)	31.8	2.38 (1H, m); 1.38 (1H, m)		
23	28.5	1.17 (3H, s)	28.6	1.18 (3H, s)		
24	17.2	1.05 (3H, s)	17.1	1.05 (3H, s)		
25	16.1	0.82 (3H, s)	16.1	0.85 (3H, s)		
26	17.5	0.90 (3H, s)	17.4	0.91 (3H, s)		
27	27.7	1.84 (3H, s)	28.1	1.83 (3H, s)		
28	69.9	4.54 (1H, d, 10.4); 3.70 (1H, d, 10.4)	70.5	3.72 (1H, d, 10.6); 3.48 (1H, d, 10.6)		
29	28.5	1.34 (3H, s)	24.8	1.03 (3H, s)		
30	67.9	4.06 (1H, d, 10.4); 3.95 (1H, d, 10.4)	208.2	9.80 (1H, s)		
5-OMe					55.7	3.66 (3H, s)
Glc-1'					105.4	5.53 (1H, d, 7.3)
2'					75.8	4.29 (1H, m)
3'					78.1	4.30 (1H, m)
4'					71.4	4.32 (1H, m)
5'					78.3	3.86 (1H, m)
6'					62.5	4.40 (1H, m); 4.32 (1H, m)
Ara-1'	104.6	4.80 (1H, d, 5.8)	104.9	4.96 (1H, brs)		

Continued

No.	1		2		3	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2'	80.1	4.50 (1H, m)	81.3	4.60 (1H, m)		
3'	73.7	4.21 (1H, m)	75.1	4.24 (1H, m)		
4'	78.3	4.25 (1H, m)	75.4	4.25 (1H, m)		
5'	64.6	3.98 (1H, m)	64.2	4.09 (1H, m)		
Glc-1"	105.3	5.53 (1H, d, 7.6)	106	5.40 (1H, d, 7.4)		
2"	76.6	4.11 (1H, m)	76.8	4.12 (1H, m)		
3"	78.7	4.35 (1H, m)	77.8	4.32 (1H, m)		
4"	72.2	4.29 (1H, m)	72.3	4.25 (1H, m)		
5"	78.6	4.08 (1H, m)	78.6	4.10 (1H, m)		
6"	63.4	4.56 (1H, m); 4.38 (1H, m)	63.4	4.79 (1H, m); 4.40 (1H, m)		
Glc-1"'	105.1	5.02 (1H, d, 7.8)	103.6	5.29 (1H, d, 6.1)		
2"'	85.8	3.90 (1H, m)	78.6	4.09 (1H, m)		
3"'	77.9	4.29 (1H, m)	80.1	4.22 (1H, m)		
4"'	71.1	4.24 (1H, m)	72.4	4.21 (1H, m)		
5"'	79.0	4.07 (1H, m)	78.9	3.82 (1H, m)		
6"'	62.7	4.47 (1H, m); 4.31 (1H, m)	63.1	4.32 (1H, m); 3.78 (1H, m)		
Xyl-1""	108.1	4.92 (1H, d, 6.5)				
2""	76.5	4.08 (1H, m)				
3""	78.2	3.95 (1H, m)				
4""	71.1	4.21 (1H, m)				
5""	67.6	4.60 (1H, m)				
Rha-1""			102	6.45 (1H, brs)		
2""			72.9	4.32 (1H, m)		
3""			73.2	4.30 (1H, m)		
4""			75.4	4.30 (1H, m)		
5""			69.9	5.06 (1H, m)		
6""			19.4	1.84 (1H, d, 5.0)		

powder. The molecular formula was determined to be $C_{20}H_{30}O_{10}$ by HR-ESI-MS (*m/z* 453.1734, $[M + Na]^+$, Calcd. for $C_{20}H_{30}NaO_{10}$, 453.1731). The ^1H NMR spectrum (Table 1) of **3** showed 1,3,4,5-tetr-asubstituted benzene ring signals at δ_H 6.74 (1H, d, J = 2.6 Hz) and 6.80 (1H, d, J = 2.6 Hz), one methoxyl proton signals at δ_H 3.66 (3H, s, 5-OCH_3), a hydroxyl anomeric H-atom signal at δ_H 11.30 (1H, brs, 3-OH). The position of methoxyl proton signals was determined at C-5 by HMBC (Fig. 2) long-range correlations between H-6 (δ_H 6.80) with C-2 (δ_C 137.3), C-5 (δ_C 155.6), and C-7 (δ_C 26.4). In addition, one *n*-butyl ester moiety signals at δ_H 0.73 (3H, t, J = 7.4 Hz, H-13), 1.17 (2H, m, H-12), 1.42 (2H, m, H-11) and 4.00 (2H, t, J = 6.7 Hz, H-10). ^{13}C NMR spectral data of **3** were similar to those of the known compound methyl 3-(2-*O*-*β*-D-glucopyranosyl-3-hydroxy-5-methoxyphenyl) propionate [15], except for the *n*-butyl ester instead of *n*-methyl ester in compound **3**. The position of the *n*-butyl ester moiety was determined at C-9 since the observation of HMBC (Fig. 2) correlations from H-13 (δ_H 0.73) to C-12 (δ_C 19.1), from H-12 (δ_H 1.17) to C-11 (δ_C 30.6), from H-11 (δ_H 1.42) to C-10 (δ_C 63.9), and from H-10 (δ_H 4.00) to C-9 (δ_C 173.4).

The sugar of compound **3** was identified as glucose acid hydrolysis, followed by co-TLC (*n*-BuOH−HOAc−H_2O, 4 ∶ 1 ∶ 1) comparsion with a standard sugar. The configurations of the anomeric positions of the glucose moieties were assigned as *β* from the coupling constants of the anomeric proton signals at δ_H 5.53 (1H, d, J = 7.3 Hz), and HMBC correlations were observed between H-1′ (δ_H 5.53) and C-2 (δ_C

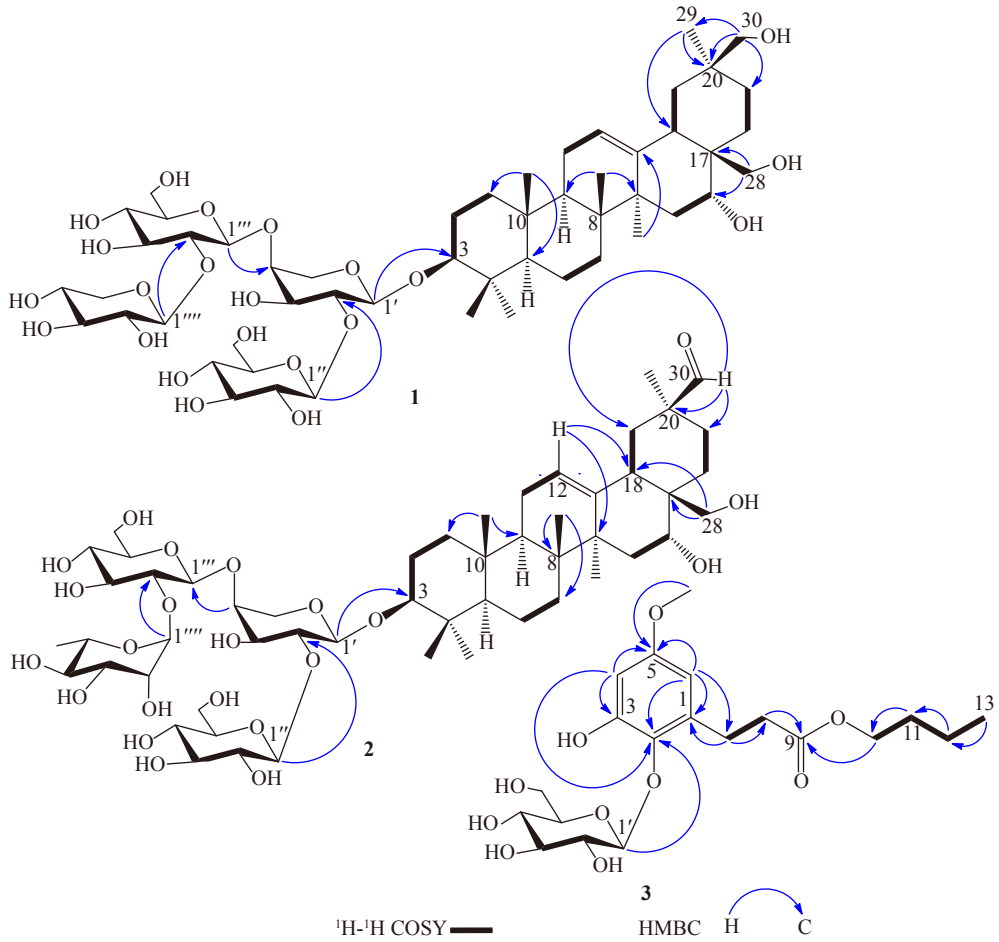

Fig. 2 Key ^{1}H–^{1}H COSY and HMBC correlations of compounds 1–3

137.3). Therefore, the structure of compound **3** was elucidated as butyl 3-(2-*O*-*β*-D-glucopyranosyl-3-hydroxy-5-methoxyphenyl) propionate. This is a new phenylpropanoid glycoside, trivially named ardicrephenin.

The known compounds (**4**–**8**) were identified as cyclamiretin A (**4**) [16], ardisicrenoside A (**5**) [17], ardicrenin (**6**) [18], cyclaminorin (**7**) [19], and ardisimamilloside H (**8**) [20] by comparison of spectral data with those reported in the literature.

Compounds **2**–**7** were evaluated for their cytotoxic activities against four human cancer cell lines (A549, MCF-7, HepG2, and MDA-MB-231) by MTT assay [21]. Among them, compounds **5** exhibited effective cytotoxicity against human breast cancer cell line MCF-7 with IC_{50} value of 5.60 ± 0.54 μmol·L^{-1}. Compound **6** showed significant cytotoxicity against A549, MCF-7, and HepG2 tumor cell lines with IC_{50} value of 1.17 ± 0.01, 1.19 ± 0.06, and 3.52 ± 0.23 μmol·L^{-1}, respectively. Compounds **2-4** and **7**, exhibited no cytotoxic activities against these cell lines with IC_{50} > 100 μmol·L^{-1}. The above results suggested that aglycone and a sugar chain at C-3 might be important for the cytotoxic activity. More extensive studies are needed before a clear structure-activity relationship can be reached. The preliminary cytotoxic activity of all the tested compounds is presented in Table 2.

Experimental

General experimental procedures

Optical rotations were determined using a JACSO P-1020 digital polarimeter (Tokyo, Japan). HR-ESI-MS were obtained using an Agilent 1100 series LC/MSD ion trap mass spectrometer (Santa Clara, USA). NMR experiments were performed on Bruker AV500-III spectrometer (Karlsruhe, Germany), using TMS as an internal standard. Preparative HPLC was performed on a Shimadzu LC-20AD instrument (Tokyo, Japan) equipped with an a SPD-10A detector using a YMC-Pack ODS-C_{18} column (20 mm × 250 mm, YMC, Tokyo, Japan). Silica gel (200−300 mesh, Qingdao Marine Chemical Co., Ltd., Qingdao, China), Sephadex LH-20 (Amersham Pharmacia Biotech, Uppsala, Sweden), and ODS (50 μm, YMC, Tokyo, Japan) were used for column chromatography. TLC was carried out with GF_{254} plates (Qingdao Marine Chemical Co., Ltd., Qingdao, China). Spots were visualized by spraying with 10% H_2SO_4 acid in EtOH followed by heating. GC was conducted on an Agilent 7890A instrument (Santa Clara, USA).

Plant materials

The roots of *Ardisia crenata* were collected from Guangxi Province, China, in September 2015, and identified

Table 2 Cytotoxic activities of compounds 2–7 against human cancer cell lines (mean ± SD, *n* = 3)

Compounds	Cell lines (IC_{50} μmol·L^{-1})			
	A549	MCF-7	HepG2	MDA-MB-231
2	> 100	> 100	> 100	> 100
3	> 100	> 100	> 100	> 100
4	> 100	> 100	> 100	> 100
5	11.42 ± 0.02	5.60 ± 0.54	28.03 ± 1.18	29.07 ± 2.89
6	1.17 ± 0.01	1.19 ± 0.06	3.52 ± 0.23	16.61 ± 1.02
7	> 100	> 100	> 100	> 100
Paclitaxel [a] (nmol·L^{-1})	5.38 ± 0.25	5.07 ± 0.07	8.44 ± 0.83	16.61 ± 0.77

[a] Paclitaxel was used as a positive control for cytotoxic assay.

by Professor QI Jin (China Pharmaceutical University). A voucher specimen (No. 2015003) was deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University, Nanjing, China.

Extraction and isolation

The air-dried and powdered roots parts of *A. crenata* (14 kg) were extracted with 95% EtOH (4 × 50 L) and further refluxed for 4 h. The combined EtOH extracts were concentrated to dryness. The residue was suspended in H_2O and then individually partitioned with petroleum ether, EtOAc, and *n*-BuOH, successively. The *n*-BuOH-soluble residue (120 g) was subjected to macroporous adsorptive resin and eluted with water and 30%, 50%, 70%, 90%, and 100% EtOH. The 50% and 70% EtOH elutions were combined and gave a total saponin fraction Fr. D (47.1 g). This was separated by silica gel column, eluted with a gradient of CH_2Cl_2–MeOH solvent system (5 : 1, 3 : 1, 1 : 1, 0 : 100, *V/V*) to obtain five main subfractions (Frs. D1–D5). Fractions D1 (20.8 g) was subjected to ODS gel column chromatography and eluted with MeOH–H_2O (40 : 60, 50 : 50, 60 : 40, 70 : 30, 100 : 0, *V/V*) to afford five subfractions (Frs. D1.1–D1.5). Fractions D1.1 was subjected to ODS gel column chromatography and eluted with MeOH–H_2O (30 : 70, 40 : 60, 45 : 55, *V/V*) to afford compound **5** (110 mg). Fraction D1.2 was subjected by repeated Sephadex LH-20 column chromatography with MeOH as mobile phase to afford compound **8** (11 mg). Fraction D2 (5.2 g) was purified by silica gel CC to afford three subfractions (Frs. D2.1–D2.3), the fraction D2.1 was further separated by preparative HPLC (MeOH–H_2O, 65 : 35, *V/V*, 10 mL·min^{-1}) as mobile phase to yield compound **6** (6 mg, t_R = 17.5 min) and compound **7** (25 mg, t_R = 38.2 min). Fraction D4 (13.2 g) was subjected to ODS gel column chromatography and eluted with MeOH–H_2O (40 : 60, 60 : 40, 100 : 0, *V/V*) to afford three subfractions (Frs. D4.1–D4.3). Fraction D4.1 was subjected to preparative HPLC (MeOH–H_2O, 45 : 55, *V/V*, 10 mL·min^{-1}) to give compound **1** (10 mg, t_R = 27.6 min). Fraction D4.2 was also purified by preparative HPLC (MeOH–H_2O, 55 : 45, *V/V*, 10 mL·min^{-1}) to afford compound **2** (16 mg, t_R = 25.1 min). Fraction D5 (2.63 g) was further separated by preparative HPLC (MeOH–H_2O) to afford compound **3** (11 mg, t_R = 31.0 min, 40% MeOH), compound **4** (5 mg, t_R = 22.5 min, 60% MeOH).

Identification of compounds

Ardisicrenoside R (**1**): a white amorphous powder; $[\alpha]_D^{20}$ +2.22 (*c* 0.05, MeOH), IR (KBr) ν_{max}: 3398, 2924, 1627, 1044 cm^{-1}; HR-ESI-MS *m/z* 1080.5940, $[M + NH_4]^+$ (Calcd. for $C_{52}H_{90}NO_{22}$, 1080.5949); ^1H (Pyridine-d_5, 600 MHz) and ^{13}C NMR (Pyridine-d_5, 150 MHz) spectral data are shown in Table 1.

Ardisicrenoside S (**2**): a white amorphous powder; $[\alpha]_D^{20}$ −5.2 (*c* 0.08, MeOH), IR (KBr) ν_{max}: 3295, 1712 cm^{-1}; HR-ESI-MS *m/z* 1097.5495, $[M + Na]^+$ (Calcd. for $C_{53}H_{86}O_{22}Na$, 1097.5503); ^1H (Pyridine-d_5, 600 MHz) and ^{13}C NMR (Pyridine-d_5, 150 MHz) spectral data are shown in Table 1.

Ardicrephenin (**3**): a yellow amorphous powder; UV (MeOH) λ_{max}: 233, 280 nm; IR (KBr) ν_{max}: 3745, 2958, 1713, 1678, 1629, 1501, 1467, 1366 cm^{-1}; HR-ESI-MS *m/z* 453.1734, $[M + Na]^+$ (Calcd. for $C_{20}H_{30}O_{10}Na$, 453.1731); ^1H (Pyridine-d_5, 600 MHz) and ^{13}C NMR (Pyridine-d_5, 150 MHz) spectral data are shown in Table 1.

*Acid hydrolysis and GC analysis of compounds **1** and **2***

Each compound (4 mg) was hydrolyzed with 2 mol·L^{-1} HCl–MeOH (5 mL) under reflux for 3 h. The reaction mixture was diluted with H_2O and then extracted with $CHCl_3$. The aqueous layer was neutralized with Na_2CO_3, and then concentrated and dried by N_2. Monosaccharide standards (2 mg) and the hydrolyzed polysaccharide (2 mg) were reacted with 10 mg $NaBH_4$–MeOH (2 mL), stirred at 25 °C for 1 h. Slowly add CH_3COOH to the reaction solution without bubbles and dry. MeOH (1 mL) was added to the residue, and then dried. Methylimidazole (400 μL) and acetic anhydride (12.5 μL) were added to the dried products and reacted for 5 min at room temperature. The reaction solution followed by partitioning between H_2O (3 mL) and EtOAc (1 mL) two times. The supernatant was evaporated to dryness and further dilute to 0.5 mL with ethyl acetate for GC analysis [22].

The GC conditions was as followed: Agilent HP-5MS (30 m × 0.25 mm × 0.25 μm); temperature program, 140–215 °C, 5 °C·min^{-1}; 215–260 °C, 2 °C·min^{-1}; carrier gas, N_2 100 kpa, H_2 50 kpa, O_2 25 kpa; detector, FID; injection volume, 1 μL. The absolute configurations of the monosaccharides were confirmed to be L-Rhamnose, L-Arabinose, D-xylose and D-glucose, which was identified by the retention times comparison with monosaccharide derivatives of standard samples: L-Rhamnose (14.3 min), L-Arabinose (14.6 min), D-xylose (15.0 min), D-glucose (20.2 min).

Cytotoxicity assay

Human lung cancer cell line A549, human breast cancer cell lines MCF-7 and MDA-MB-231, human hepatocellular carcinoma cell line HepG2 were all obtained from the Cell Bank of Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. A549 cells were cultured in RPMI-1640 medium (Gibco, Invitrogen Corporation, NY)

supplemented with 10% fetal bovine serum (Royal, Lanzhou, China), 100 U·mL^{-1} benzyl penicillin, and 100 U·mL^{-1} streptomycin in a humidified environment with 5% CO_2 at 37 °C. MCF-7, HepG2, and MDA-MB-231 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Invitrogen Corporation, NY) supplemented with 10% fetal bovine serum (Royal, Lanzhou, China), 100 U·mL^{-1} benzyl penicillin, and 100 U·mL^{-1} streptomycin in a humidified environment with 5% CO_2 at 37 °C.

The cytotoxic activities of compounds **2**−**7** against human cancer cell lines A549, MCF-7, HepG2 and MDA-MB-231 were preliminary evaluated by MTT assay, and paclitaxel was used as a positive control. Experiments were carried out in triplicate in a parallel manner. Negative control cells were treated with culture media containing 0.1% DMSO. After incubation at 37 °C for 24 h, absorbance (A) was measured at 570 nm. Cell viability (%) was calculated using the following equation: cell viability (%) = ($A_{treatment}$/$A_{control}$) × 100. IC_{50} (the concentration that caused 50% inhibition of cell proliferations) was calculated (see Table 2).

Acknowledgement

The authors are grateful to CHEN Dong-Jun and QI Jin for obtaining the spectra and identificating plants, respectively. Gas chromatograph service provided by YU Qing-Feng is grateful acknowledged.

References

[1] Jiangsu New Medical College. *Dictionary of Chinese traditional medicine* [M]. Shanghai Science and Technology Press, 1986: 2358.
[2] Liu DL, Wang NL, Zhang X, *et al*. Three new triterpenoid saponins from *Ardisia crenata* [J]. *Helv Chim Acta*, 2011, **94**(4): 693-702.
[3] Liu DL, Zhang X, Zhao YM, *et al*. Three new triterpenoid saponins from the roots of *Ardisia crenata* and their cytotoxic activities [J]. *Nat Prod Res*, 2016, **30**(23): 2694-2703.
[4] Li M, Wei SY, Xu B, *et al*. Pro-apoptotic and microtubule-disassembly effects of ardisiacrispin (A+B), triterpenoid saponins from *Ardisia crenata* on human hepatoma Bel-7402 cells [J]. *J Asian Nat Prod Res*, 2008, **10**(8): 729-736.
[5] Liu DL, Wang NL, Zhang X, *et al*. Two new triterpenoid saponins from *Ardisia crenata* [J]. *J Asian Nat Prod Res*, 2007, **9**(2): 119-127.
[6] Sonia P. Constituents of *Ardisia japonica* and their *in vitro* anti-HIV activityanti-HIV activity [J]. *J Nat Prod*, 1996, **59**(6): 565-569.
[7] Li YY, Li K, Wang JX, *et al*. α-Glucosidase inhibitory and antioxidant activity of *Ardisia crenata* [J]. *Nat Prod Res Dev*, 2012, **24**(9): 1257-1260.
[8] Chang XL, Li W, Jia ZH, *et al*. Biologically active triterpenoid saponins from *Ardisia japonica* [J]. *J Nat Prod*, 2007, **70**(2): 179-187.
[9] Koike K, Jia ZH, Ohura S, *et al*. Minor triterpenoid saponins from *Ardisia crenata* [J]. *Chem Pharm Bull*, 1999, **47**(3): 434-435.
[10] Liu DL, Zhang X, Wang SP, *et al*. A new triterpenoid saponin from the roots of *Ardisia crenata* [J]. *Chin Chem Lett*, 2011, **22**(8): 957-960.
[11] Zheng ZF, Xu JF, Feng ZM, *et al*. Cytotoxic triterpenoid saponins from the roots of *Ardisia crenata* [J]. *J Asian Nat Prod Res*, 2008, **10**(9): 833-839.
[12] Huang J, Oglihara Y, Zhang H, *et al*. Ardisimamillosides C-F, four new triterpenoid saponins from *Ardisia mamillata* [J]. *Chem Pharm Bull*, 2000, **48**(10): 1413-1417.
[13] Pal BC, Roy G, Mahato SB. Triterpenoid sapogenols from *Androsace saxifragifolia*: The structure of androsacenol [J]. *Phytochemistry*, 1984, **23**(7): 1475-1479.
[14] Zou YP, Tan CH, Wang BD, *et al*. Phenolic compounds from *Ranunculus chinensis* [J]. *Chem Nat Compd*, 2010, **46**(1): 19-21.
[15] Gao YY, Zeng P, Jia CL, *et al*. Two new phenols from *Lysimachia patungensis* [J]. *J Asian Nat Prod Res*, 2017, **19**(1): 28-34.
[16] Zhang QH, Huang SL, Wang XJ. Studies of sapogenins and prosapogenins in *Ardisia pusilla* A.DC [J]. *Chin J Chin Mater Med*, 1993, **18**(9): 545-547.
[17] Jia ZH, Koike K, Ohmoto T, *et al*. Triterpenoid saponins from *Ardisia crenata* [J]. *Phytochemistry*, 1994, **37**(5): 1389-1396.
[18] Ma Y, Pu SR, Cheng QS, *et al*. Isolation and characterization of ardicrenin from *Ardisia crenata* Sims [J]. *Plant Soil Environ*, 2009, **55**(7): 305-310.
[19] Calis I, Yürüker A, Tanker N, *et al*. Triterpene saponins from *Cyclamen coum* var. *coum*. [J]. *Planta Med*, 1997, **63**(2): 166-170.
[20] Huang J, Zhang H, Shimizu N, *et al*. Ardisimamillosides G and H, two new triterpenoid saponins from *Ardisia mamillata* [J]. *Chem Pharm Bull*, 2003, **51**(7): 875-877.
[21] Liu W, Ning R, Chen RN, *et al*. Aspafilioside B induces G_2/M cell cycle arrest and apoptosis by up-regulating H-Ras and N-Ras *via* ERK and p38 MAPK signaling pathways in human hepatoma HepG2 cells [J]. *Mol Carcinog*, 2016, **55**(5): 440-457.
[22] Wu J, Li MH, Lin JP, *et al*. Determination of dihydroxyacetone and glycerol in fermentation process by GC after *n*-methylimidazole catalyzed acetylation [J]. *J Chromatogr Sci*, 2011, **49**(5): 375-378.

Cite this article as: SONG Ning-Ning, YANG Lei-Min, ZHANG Min-Jie, AN Ren-Feng, LIU Wei, HUANG Xue-Feng. Triterpenoid saponins and phenylpropanoid glycoside from the roots of *Ardisia crenata* and their cytotoxic activities [J]. *Chin J Nat Med*, 2021, **19**(1): 63-69.