Three New Triterpenoid Saponins from *Ardisia crenata*

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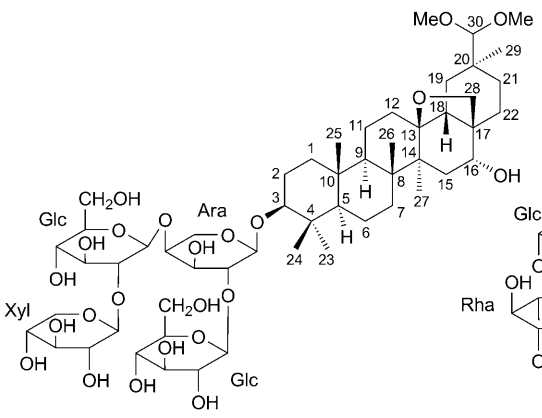
Three new triterpenoid saponins, ardisicrenoside I (**1**), ardisicrenoside J (**2**), and ardisicrenoside M (**3**), along with eight known compounds, were isolated from the roots of *Ardisia crenata* SIMS. Their structures were elucidated as 16α-hydroxy-30,30-dimethoxy-3β-*O*-{β-D-xylopyranosyl-(1$\rightarrow$2)-β-D-glucopyranosyl-(1$\rightarrow$4)-[β-D-glucopyranosyl-(1$\rightarrow$2)]-α-L-arabinopyranosyl}-13β,28-epoxyoleanane (**1**), 16α-hydroxy-30,30-dimethoxy-3β-*O*-{α-L-rhamnopyranosyl-(1$\rightarrow$2)-β-D-glucopyranosyl-(1$\rightarrow$4)-[β-D-glucopyranosyl-(1$\rightarrow$2)]-α-L-arabinopyranosyl}-13β,28-epoxyoleanane (**2**), 30,30-dimethoxy-16-oxo-3β-*O*-{β-D-xylopyranosyl-(1$\rightarrow$2)-β-D-glucopyranosyl-(1$\rightarrow$4)-[β-D-glucopyranosyl-(1$\rightarrow$2)]-α-L-arabinopyranosyl}-13β,28-epoxyoleanane (**3**), ardisiacrispin A (**4**), ardisiacrispin B (**5**), ardisicrenoside B (**6**), ardisicrenoside A (**7**), ardisicrenoside H (**8**), ardisicrenoside G (**9**), cyclamiretin A-3β-*O*-β-D-xylopyranosyl-(1$\rightarrow$2)-β-D-glucopyranosyl-(1$\rightarrow$4)-α-L-arabinopyranoside (**10**), and cyclamiretin A-3β-*O*-α-L-rhamnopyranosyl-(1$\rightarrow$2)-β-D-glucopyranosyl-(1$\rightarrow$4)-α-L-arabinopyranoside (**11**) by means of chemical and spectral analysis, and their cytotoxicities were evaluated *in vitro*.

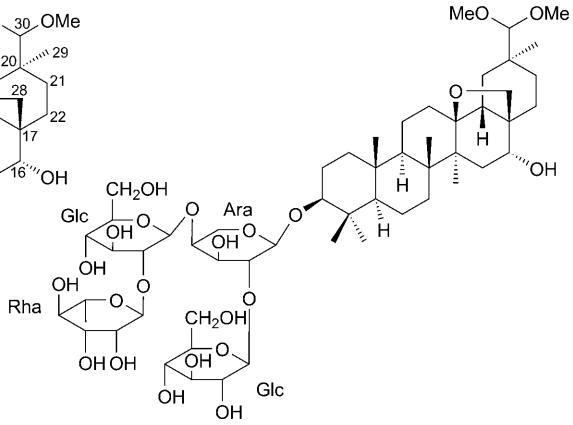
Introduction. – *Ardisia* is a large genus of the family Myrsinaceae, comprising more than 300 species distributed around the world. Previous chemical studies showed that triterpenoid saponins were the main components from the plants of this genus [1][2], which have showed a wide range of bioactivities: utero-contracting activity, inhibitory activity on cAMP phosphodiesterase, cytotoxicity, anti-HIV and anticancer activities, *etc.* [3–7].

The roots of *Ardisia crenata* SIMS are used as the traditional Chinese medicine '*Zhu Sha Gen*' for the treatment of respiratory tract infections and menstrual disorders in China [8]. We have reported several antifungal triterpenoid saponins from this plant in our previous study [7]. The EtOH extract of *Ardisia crenata* SIMS also showed good cytotoxic activity on the cancer cell lines in our further study. In search for further bioactive chemical constituents of this plant, three new compounds, ardisicrenoside I (**1**), ardisicrenoside J (**2**), and ardisicrenoside M (**3**), along with eight known compounds, were isolated. In this article, we report the isolation and structure elucidation of these compounds, as well as their cytotoxic activities against the MCF-7, NCI-H460, SF-268, HepG2, HepG2-r, and 293 cell lines.

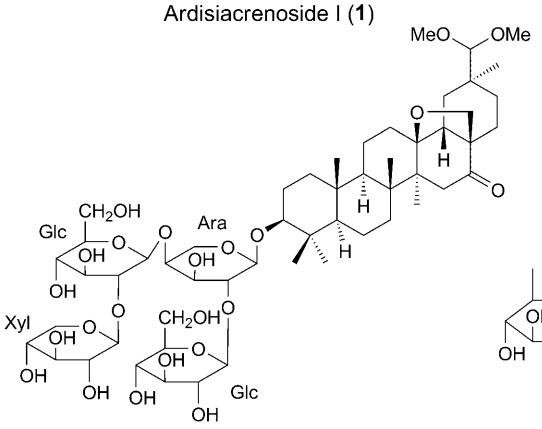
Results and Discussion. – The roots of *A. crenata* SIMS were extracted with 60% EtOH. The EtOH extract was dissolved in H_2O, and then partitioned with AcOEt and



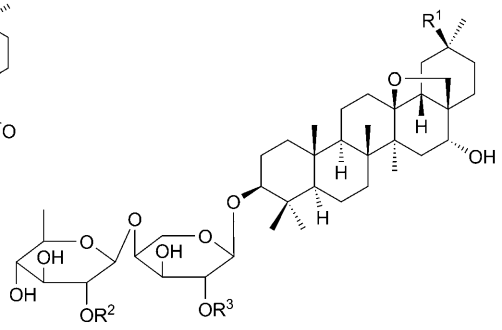
Ardisiacrenoside I (**1**)



Ardisiacrenoside J (**2**)



Ardisiacrenoside M (**3**)



	R^1	R^2	R^3
4	CHO	Xyl	Glc
5	CHO	Rha	Glc
6	HO–CH_2	Xyl	Glc
7	HO–CH_2	Rha	Glc
8	COOH	Xyl	Glc
9	COOH	Rha	Glc
10	CHO	Xyl	H
11	CHO	Rha	H

BuOH. The BuOH-soluble portion was subjected to column chromatography on *Diaion HP-20* and *MPLC RP-18*. The saponin-containing fractions were further purified by repeated HPLC on *ODS* column, to afford three new triterpenoid saponins, ardisicrenosides I–M (**1**–**3**, resp.), together with eight known compounds, were isolated.

Compound **1**, a white powder, showed positive to *Libermann–Burchard*'s reaction and *Molish* test. The HR-ESI mass spectrum (positive-ion mode) revealed the *quasi*-molecular-ion peak at *m/z* 1129.5822 ($[M+Na]^+$), corresponding to the molecular formula $C_{54}H_{90}O_{23}$. The ^{1}H-NMR spectrum of compound **1** in (D_5)pyridine exhibited signals of the six tertiary Me groups at δ(H) 1.56, 1.36, 1.26, 1.21, 1.09, and 0.87, and two

MeO groups at δ(H) 3.54 (*s*) and 3.52 (*s*), and four anomeric H-atom signals of sugar moieties at δ(H) 5.48 (*d*, $J = 7.6$), 4.98 (*d*, $J = 7.7$), 4.92 (*d*, $J = 7.3$), and 4.78 (br. *s*). The above data suggested that ardisicrenoside I contains four sugars moieties and a triterpenoid aglycone. Complete analysis of the ^{13}C-NMR and DEPT spectra of compound **1** revealed 54 C-atom signals, 32 of them were assigned to the aglycone part and 22 to the sugar moiety. ^{13}C-NMR Data of compound **1** were similar to those of the known saponin ardisiacrispin A [9]. As listed in *Table 1*, there was a signal at δ(C) 108.7 (CH, by DEPT) of compound **1** instead of a signal at δ(C) 207.2 due to the 30-CHO group of ardisiacrispin A. Furthermore, in the HMBC spectrum of compound **1**, the C-atom signal at δ(C) 108.7 not only correlated with Me(29) but also with the two MeO signals, respectively, confirming that δ(C) 108.7 was the signal of C(30), and the two MeO groups were located at C(30). The O-bearing groups at C(3) and C(16) in the aglycone were deduced from the signals at δ(C) 88.9 and 76.9, respectively. The configuration at C(16) was determined using a NOESY experiment. NOE Correlations of the signals at δ(H) 3.17 (H_{ax}–C(3)) with those at 1.21 (Me(23)) and 0.70 (H–C(5)), and of the signal at δ(H) 4.24 (H–C(16)) with that at 3.41 (CH_2(28)), indicate an α-orientation for the H-atom at C(3) and a β-orientation for the H-atom at C(16). The α-orientation of OH at C(16) was determined by comparing the chemical shift of C(16) (δ(C) 77.3) with that in the literature (δ(C) 77.0; HO_β–C(16), δ(C) 64.0) [2]. From the above evidences, the structure of the new sapogenin was established as 3β,16α-dihydroxy-30,30-dimethoxy-13β,28-epoxyoleanane.

On acid hydrolysis of compound **1**, arabinose, glucose, and xylose were identified by co-TLC with authentic samples. The ESI mass spectrum (positive-ion mode) exhibited a single predominant peak at *m*/*z* 1129 ($[M + Na]^+$), giving rise to the peaks at *m*/*z* 997 ($[M + Na - 132]^+$) and 835 ($[M + Na - 132 - 162]^+$) in its ESI-MS^2. The peak at *m*/*z* 835 gave a prominent ion peak at *m*/*z* 673 ($[M + Na - 132 - 162 \times 2]^+$) in the ESI-MS^3. In combination with the ESI-MS results, it could be concluded that the ratio of arabinose, glucose, and xylose in the molecule of compound **1** was 1 : 2 : 1. ^{1}H,^{1}H-COSY, TOCSY, HMBC, and NOESY analyses were used to determine the sequence of the oligosaccharide chain in **1**. From the relatively large anomeric H–C(1) coupling constants (7.6, 7.7, and 7.3 Hz), both glucose moieties and the xylose moiety should be β-configured [10]. The small H–C(1) coupling constant of arabinose indicated that it was possess α-oriented. Based on these results and comparison of the signals of the sugars moieties of compound **1** in the ^{13}C-NMR spectrum with those in the literature [7][9], the four sugars and their anomeric configurations in **1** were determined as an α-L-arabinopyranose, two β-D-glucopyranoses, and a β-D-xylopyranose. The sequence of the oligosaccharide chain was deduced by comparing the chemical shifts of their individual sugar residues with those of published compounds [10], and confirmed by HMBC and NOESY experiments. The C(1) of arabinose was attached to C(3)–O of the aglycone, as indicated by the correlation between the anomeric H-atom signal at δ(H) 4.78 (Ara H–C(1)) and the signal at δ(C) 88.9 (C(3)) in HMBC spectrum, and as well as with the signal at δ(H) 3.17 (H–C(3)) in NOESY spectrum. From the HMBC of **1** (*Fig.*), the following correlations were also observed: δ(H) 5.48 (terminal Glc H–C(1))/δ(C) 79.7 (Ara C(2)), δ(H) 4.98 (inner Glc H–C(1))/δ(C) 78.6 (Ara C(4)), and δ(H) 4.92 (Xyl H–C(1))/δ(C) 85.4 (inner Glc C(2)). On the basis of the analyses above, compound **1** was identified to be 16-hydroxy-30,30-dimethoxy-3β-*O*-{β-D-

Table 1. *^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) Data for* **1**, **2**, *and* **3** *in C_5D_5N. δ* in ppm, *J* in Hz.

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	1.70[a]), 0.94[a])	39.2 (*t*)	1.69[a]), 0.90[a])	39.2 (*t*)	1.66[a]), 0.89[a])	39.0 (*t*)
2	2.03[a]), 1.85[a])	26.6 (*t*)	1.87[a]), 2.04[a])	26.5 (*t*)	1.99[a]), 1.81[a])	26.5 (*t*)
3	3.17 (*dd*, *J* = 11.5, 4.5)	88.9 (*d*)	3.16 (*dd*, *J* = 11.6, 4.2)	89.1 (*d*)	3.17 (*dd*, *J* = 11.8, 4.4)	88.8 (*d*)
4		39.7 (*s*)		39.6 (*s*)		39.7 (*s*)
5	0.70 (*d*, *J* = 8.3)	55.7 (*d*)	0.66 (*d*, *J* = 11)	55.6 (*d*)	0.66 (*d*, *J* = 8.6)	55.5 (*d*)
6	1.44[a]), 1.41[a])	17.9 (*t*)	1.40[a]), 1.39[a])	17.9 (*t*)	1.42[a]), 1.35[a])	17.7 (*t*)
7	2.08[a]), 1.26[a])	34.4 (*t*)	1.52[a]), 1.22[a])	34.4 (*t*)	1.34[a]), 1.02[a])	33.7 (*t*)
8		42.5 (*s*)		42.4 (*s*)		42.9 (*s*)
9	1.29[a])	50.4 (*d*)	1.25[a])	50.4 (*d*)	1.16 (*d*, *J* = 10.7, 1 H)	50.1 (*d*)
10		36.9 (*s*)		36.8 (*s*)		36.7 (*s*)
11	1.81[a]), 1.47[a])	19.2 (*t*)	1.48[a]), 1.28[a])	19.2 (*t*)	1.78[a]), 1.49[a])	18.9 (*t*)
12	2.16[a]), 1.53[a])	33.1 (*t*)	2.18[a]), 1.54[a])	33.0 (*t*)	1.97[a]), 1.57[a])	31.9 (*t*)
13		86.6 (*s*)		86.6 (*s*)		86.3 (*s*)
14		44.6 (*s*)		44.6 (*s*)		49.8 (*s*)
15	2.70[a]), 1.49[a])	36.9 (*t*)	2.26 (*dd*, *J* = 14, 4.1), 1.50[a])	36.9 (*t*)	2.86 (*d*, *J* = 16.1), 2.01[a])	45.7 (*t*)
16	4.24[a])	76.9 (*d*)	4.26[a])	77.0 (*d*)		212.6 (*s*)
17		44.4 (*s*)		44.4 (*s*)		55.9 (*s*)
18	1.83[a])	50.8 (*d*)	1.83[a])	50.8 (*d*)	2.19 (*d*, *J* = 14.8)	53.9 (*d*)
19	2.73 (*dd*, *J* = 14.3), 1.56[a])	34.2 (*t*)	2.73[a]), 2.08[a])	34.2 (*t*)	2.13 (*d*, *J* = 12.8), 1.38 (*m*)	35.8 (*t*)
20		40.9 (*s*)		40.9 (*s*)		40.6 (*s*)
21	2.53 (*ddd*, *J* = 14.3, 5.3), 1.98[a])	32.3 (*t*)	2.52 (*ddd*, *J* = 5.3, 1.8), 1.96[a])	32.3 (*t*)	1.87 (*m*), 1.80 (*m*)	31.5 (*t*)
22	1.99[a]), 1.70[a])	31.6 (*t*)	1.99[a]), 1.75[a])	31.6 (*t*)	2.35 (*d*, *J* = 13.2), 1.37[a])	24.9 (*t*)
23	1.21 (*s*)	28.0 (*q*)	1.16 (*s*)	27.9 (*q*)	1.22 (*s*)	27.9 (*q*)
24	1.09 (*s*)	16.6 (*q*)	1.03 (*s*)	16.5 (*q*)	1.07 (*s*)	16.6 (*q*)
25	0.87 (*s*)	16.4 (*q*)	0.87 (*s*)	16.4 (*q*)	0.83 (*s*)	16.1 (*q*)
26	1.36 (*s*)	18.5 (*q*)	1.35 (*s*)	18.5 (*q*)	1.31 (*s*)	18.7 (*q*)
27	1.56 (*s*)	19.5 (*q*)	1.55 (*s*)	19.5 (*q*)	1.09 (*s*)	21.7 (*q*)
28	3.41 (*d*, *J* = 7.4), 3.66[a])	77.8 (*t*)	3.65 (*d*, *J* = 7.4), 3.41 (*d*, *J* = 7.4)	77.8 (*t*)	3.95 (*d*, *J* = 8.3), 3.59 (*d*, *J* = 8.3)	74.9 (*t*)
29	1.26 (*s*)	24.0 (*q*)	1.25 (*s*)	24.0 (*q*)	1.04 (*s*)	23.9 (*q*)
30	4.67 (*s*)	108.7 (*d*)	4.66 (*s*)	108.7 (*d*)	4.50 (*s*)	108.0 (*d*)
(*Me*O)$_2$C(30)	3.54 (*s*), 3.52 (*s*)	58.5 (*q*), 58.4 (*q*)	3.49 (*s*), 3.48 (*s*)	58.5 (*q*), 58.3 (*q*)	3.49 (*s*), 3.48 (*s*)	58.4 (*q*), 58.3 (*q*)

Table 1 (cont.)

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
Ara						
1	4.78 (*d*, *J* = 6)	104.6 (*d*)	4.98 (br. *s*)	104.3 (*d*)	4.78 (*d*, *J* = 5.9)	104.6 (*d*)
2	4.58[a])	79.7 (*d*)	4.57[a])	80.7 (*d*)	4.56[a])	79.7 (*d*)
3	4.26[a])	73.3 (*d*)	4.49[a])	72.3 (*d*)	4.26[a])	73.2 (*d*)
4	4.22[a])	78.6 (*d*)	4.58[a])	74.7 (*d*)	4.23[a])	78.5 (*d*)
5	4.62 (*d*, *J* = 4.0), 2.27[a])	64.2 (*t*)	4.39[a]), 3.79[a])	63.5 (*t*)	4.60[a]), 3.67[a])	64.1 (*t*)
Terminal Glc						
1	5.48 (*d*, *J* = 7.6)	104.9 (*d*)	5.36 (*d*, *J* = 8.0)	105.4 (*d*)	5.49 (*d*, *J* = 7.6)	104.9 (*d*)
2	4.12[a])	76.2 (*d*)	4.06[a])	76.3 (*d*)	4.08[a])	76.2 (*d*)
3	4.01[a])	77.9 (*d*)	4.28[a])	78.0 (*d*)	4.00[a])	77.9 (*d*)
4	4.24[a])	71.8 (*d*)	4.21[a])	71.7 (*d*)	4.20[a])	71.8 (*d*)
5	4.06[a])	77.8 (*d*)	4.04[a])	78.1 (*d*)	4.03[a])	77.8 (*d*)
6	4.40[a]), 4.55[a])	62.9 (*t*)	4.46[a]), 4.38[a])	62.8 (*t*)	4.40[a]), 4.54[a])	63.0 (*t*)
Inner Glc						
1	4.98 (*d*, *J* = 7.8)	104.2 (*d*)	5.23 (*d*, *J* = 7.6)	103.1 (*d*)	5.00 (*d*, *J* = 7.8)	104.1 (*d*)
2	3.93[a])	85.4 (*d*)	4.24[a])	77.3 (*d*)	3.93[a])	85.4 (*d*)
3	4.20[a])	77.6 (*d*)	4.18[a])	79.5 (*d*)	4.21[a])	77.6 (*d*)
4	4.21[a])	71.1 (*d*)	4.12[a])	71.8 (*d*)	4.22[a])	71.1 (*d*)
5	3.80[a])	78.3 (*d*)	3.78[a])	78.3 (*d*)	3.79[a])	78.2 (*d*)
6	4.42[a]), 4.30[a])	62.3 (*t*)	4.43[a]), 4.31[a])	62.6 (*t*)	4.42[a]), 4.30[a])	62.3 (*t*)
Xyl						
1	4.92 (*d*, *J* = 7.3)	107.7 (*d*)			4.91 (*d*, *J* = 7.2)	107.7 (*d*)
2	4.03[a])	76.1 (*d*)			4.02[a])	76.1 (*d*)
3	4.01[a])	78.2 (*d*)			4.01[a])	78.3 (*d*)
4	4.17[a])	70.7 (*d*)			4.13[a])	70.6 (*d*)
5	4.53[a]), 3.70[a])	67.4 (*t*)			4.53[a]), 3.71[a])	67.4 (*t*)
Rha						
1			6.39 (*d*, *J* = 1.2)	101.5 (*d*)		
2			4.49[a])	72.3 (*d*)		
3			4.68[a])	72.7 (*d*)		
4			4.27[a])	74.8 (*d*)		
5			5.02[a])	69.4 (*d*)		
6			1.80 (*d*, *J* = 6.1)	18.9 (*q*)		

[a]) Overlapping signals are indicated. The C- and H-atom signals were unambiguously assigned by HMQC, HMBC, and TOCSY.

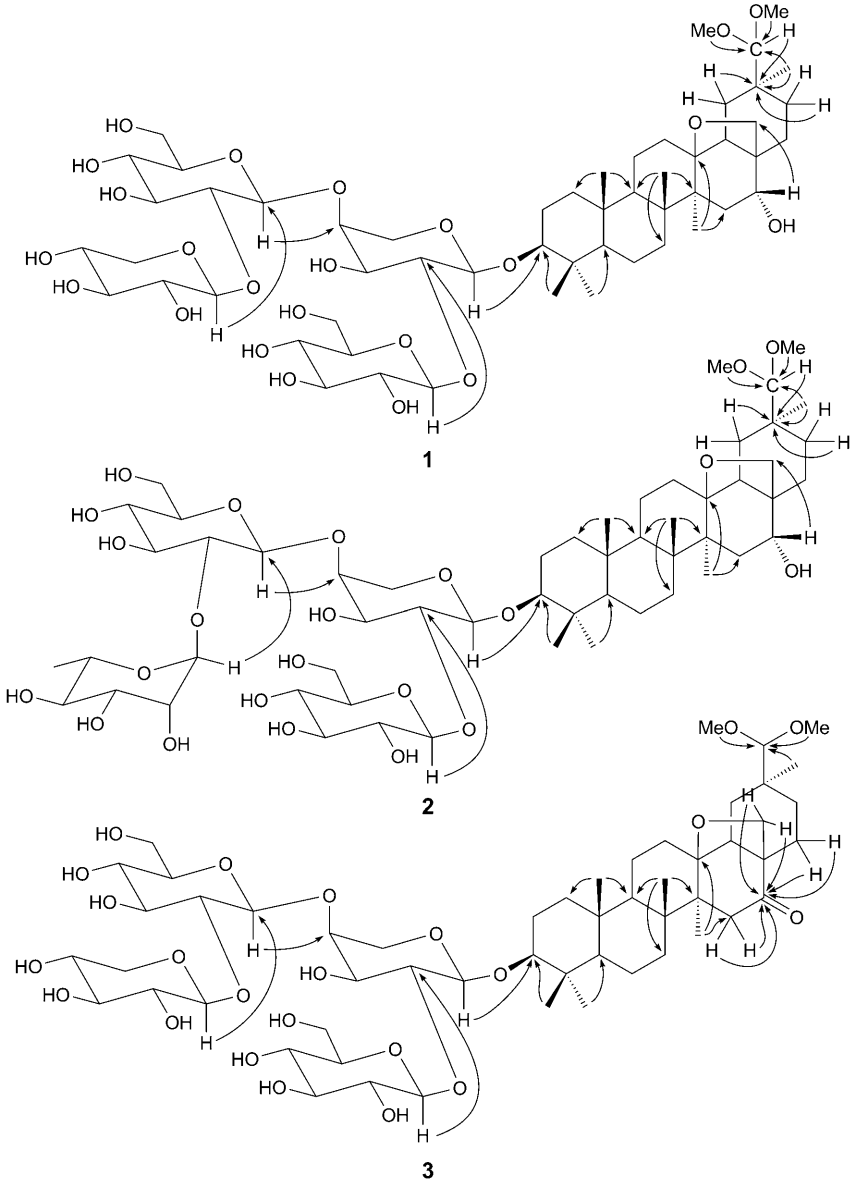


Figure. *Key HMBCs* (H → C) *for compounds* **1**, **2**, *and* **3**

xylopyranosyl-(1 → 2)-*β*-D-glucopyranosyl-(1 → 4)-[*β*-D-glucopyranosyl-(1 → 2)]-*α*-L-arabinopyranosyl}-13*β*,28-epoxyoleanane, named as ardisicrenoside I.

Compound **2** obtained as a white powder, showed positive to both the *Liebermann–Burchard* reaction and the *Molish* test. The molecular formula $C_{55}H_{92}O_{23}$ was established by HR-ESI-MS (positive-ion mode), which exhibited a *quasi*-molecular-

ion peak at *m/z* 1143.5934 ($[M+Na]^+$). The ^{1}H-NMR spectrum of compound **2** in (D_5)pyridine displayed signals for six tertiary Me groups at δ(H) 1.55, 1.35, 1.25, 1.16, 1.03, and 0.87; a Me group of rhamnose at δ(H) 1.80 (*d*, $J=6.0$); two MeO groups at δ(H) 3.49 and 3.48, and four anomeric H-atom signals from sugars at δ(H) 6.39 (br. *s*), 5.36 (*d*, $J=8.0$), 5.23 (*d*, $J=7.6$), and 4.98 (br. *s*). Its ^{1}H- and ^{13}C-NMR spectra indicated that compound **2** had the same aglycone as that of compound **1**, but differed in the oligosaccharide part (see *Table 1*). So, the structure of sapogenin of **2** is determined as 3β,16α-dihydroxy-30,30-dimethoxy-13β,28-epoxyoleanane.

The ESI mass spectrum of **2** exhibited a single predominant peak at *m/z* 1143, assigned to $[M+Na]^+$, which gave ion peaks at *m/z* 997 ($[M+Na-146]^+$) and 835 ($[M+Na-146-162]^+$) in the ESI-MS^2 (positive-ion mode). The ion peak at *m/z* 835 gave rise to the ion peak at *m/z* 673 ($[M+Na-146-2\times162]^+$) in the MS^3. The ion peak at *m/z* 1119 ($[M-H]^-$) gave ion peaks at *m/z* 973 ($[M-H-146]^-$) and 811 ($[M-H-146-162]^-$) in the ESI-MS^2 (negative-ion mode), and the MS^3 of *m/z* 811 gave a prominent ion peak at *m/z* 649 ($[M-H-146-2\times162]^-$). On acid hydrolysis, **2** afforded arabinose, glucose, and rhamnose in a ratio of 1:2:1 (analyzed by the same method as that for **1**). By comparing the vicinal coupling constant of anomeric H-atoms with those of model compounds reported in [10], the three sugars were determined as α-L-arabinopyranose, β-D-glucopyranose, and α-L-rhamnopyranose. According to the same methods as those for **1**, the HMBC cross-peaks δ(H) 4.98 (Ara H–C(1))/δ(C) 89.1 (C(3)), δ(H) 5.36 (terminal Glc H–C(1))/δ(C) 80.7 (Ara C(2)), δ(H) 5.23 (inner Glc H–C(1))/δ(C) 74.7 (Ara C(4)), and δ(H) 6.39 (Rha H–C(1))/δ(C) 77.3 (inner Glc C(2)) were observed, confirming the sugar sequence and the glycosylation position. Based on the above evidences, the structure of **2** was elucidated as 16α-hydroxy-30,30-dimethoxy-3β-*O*-{α-L-rhamnopyranosyl-(1$\rightarrow$2)-β-D-glucopyranosyl-(1$\rightarrow$4)-[β-D-glucopyranosyl-(1$\rightarrow$2)]-α-L-arabinopyranosyl}-13β,28-epoxyoleanane, named ardisicrenoside J.

Compound **3** was obtained as a white powder. The positive *Libermann–Burchard* reaction and *Molish* test suggested it to be also a triterpenoid glycoside. Its HR-ESI-MS (positive-ion mode) displayed a *quasi*-molecular-ion peak at *m/z* 1127.5682 ($[M+Na]^+$), which indicated the molecular formula $C_{54}H_{88}O_{23}$. The ^{1}H-NMR spectrum exhibited signals due to six tertiary Me groups at δ(H) 0.83, 1.04, 1.07, 1.09, 1.22, and 1.31, two MeO groups at δ(H) 3.48 and 3.49, and four anomeric H-atom signals from sugars at δ(H) 5.49 (*d*, $J=7.6$), 5.00 (*d*, $J=7.8$), 4.91 (*d*, $J=7.2$), and 4.78 (*d*, $J=5.9$). The ^{13}C-NMR spectrum showed the signals of a CO group at δ(C) 212.6, of an acetal C-atom at δ(C) 108.0, of two MeO groups at δ(C) 58.4 and 58.3, and of four anomeric C-atoms at δ(H) 107.7, 104.9, 104.6, and 104.1. All these data suggest that compound **3** contains four sugars moieties and a triterpenoid aglycone. The ^{1}H- and ^{13}C-NMR data indicated that the aglycone of **3** was the same as that of ardisicrenoside K, which we published before [7], but the oligosaccharide part was different. So the aglycone of **3** was elucidated as 3β-hydroxy-30,30-dimethoxy-16-oxo-13β,28-epoxyoleanane.

After acid hydrolysis, the sugar moieties were determined as arabinose, glucose, and xylose by TLC comparison with authentic samples. According to ESI-MS fragments analyzed by the same methods as those for **1**, it can be concluded that the ratio of arabinose, glucose, and xylose in the molecule of **3** was 1:2:1. β-Configuration at the anomeric position was inferred from the values of the coupling constants for both

glucopyranosyl units (7.8 and 7.6 Hz) and xylopyranosyl unit (7.2 Hz). Analyzed by the same methods as those for **1**, it was concluded that the anomeric position of arabinopyranosyl unit should possess α-configuration. The ^{13}C-NMR spectral data for four sugars in **3** were also in good agreement with those of **1**. By HMBC analysis, the correlations δ(H) 4.78 (Ara H–C(1))/δ(C) 88.8 (C(3)), δ(H) 5.49 (terminal Glc H–C(1))/δ(C) 79.7 (Ara C(2)), δ(H) 5.00 (inner Glc H–C(1))/δ(C) 78.5 (Ara C(4)), and δ(H) 4.91 (Xyl H–C(1))/δ(C) 85.4 (inner Glc C(2)) were observed, which confirmed the sugar sequence and the glycosylation position. Based on these data, **3** was determined as 30,30-dimethoxy-16-oxo-3β-*O*-{β-D-xylopyranosyl-(1 $\rightarrow$ 2)-β-D-glucopyranosyl-(1 $\rightarrow$ 4)-[β-D-glucopyranosyl-(1 $\rightarrow$ 2)]-α-L-arabinopyranosyl}-13β,28-epoxyoleanane and named ardisicrenoside M. Complete assignment of **3** was achieved with the aid of the ^{1}H,^{1}H-COSY, HMQC, and HMBC spectra.

The MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) method was used to test the cytotoxicity of these compounds on MCF-7, NCI-H460, SF-268, HepG2, and HepG2R cancer cell lines, and HEK 293 cell line. The bioassay results of all isolated compounds are collected in *Table 2*. From these results, it was concluded that compound **1**, **2**, **4**, **5**, and **6** exhibited very strong cytotoxicities against cancer cell lines, compound **10** and **11** had relatively weak activities against MCF-7, NCI-H460, and SF-268 cancer cell lines, compound **3** and **7** had selective inhibitory activity against NCI-H460 cell line, and compound **8** and **9** showed no activity. It is remarkable that compound **2** and **4** had very strong activity against cancer cell lines but no cytotoxicity against HEK 293 cell line.

Table 2. *The* IC_{50} *Values* [μM] *for the Inhibition of the Growth of Cancer Cell Lines*

	MCF-7	NCI-H460	SF-268	HepG2	HepG2R	HEK 293
1	14.04	14.01	9.04	16.27	9.04	15.37
2	> 22.32	> 22.32	11.34	15.18	–	–
3	–	12.36	–	–	–	–
4	4.72	> 23.58	2.36	6.60	> 23.58	> 23.58
5	5.77	12.10	3.72	4.19	3.72	8.38
6	11.21	10.87	5.40	11.21	6.29	7.39
7	–	14.51	–	–	–	–
8	–	–	–	–	–	–
9	–	–	–	–	–	–
10	24.12	13.16	> 27.41			
11	> 27.84	4.51	10.92	–	–	–

On the basis of cytotoxic activity, the triterpenoid saponins from *A. crenata* SIMS display following structure–activity relationships: the activity of **3**, with a C(16)=O group, was lower than those of **1**, **2**, **4**, and **5**. The activities of **10** and **11**, with three sugar moieties at C(3), were decreased. A C(12)=C(13) bond and a COOH group at C(30) diminished the activities of **8** and **9**. These results indicated that the sugar chain at C(3) of aglycone, the different substituents at C(30), at 16α-OH and 13,28-epoxy groups played crucial roles in the cytotoxicity.

The authors gratefully acknowledge Prof. *Qishi Sun* at the Department of Pharmacognosy, Shenyang Pharmaceutical University for identification of the plant, and Prof. *Mengsu Yang* and his staff at the Department of Biology and Chemistry, City University of Hong Kong, for the bioactivity assay of

compounds. Thanks are also extended to the *Shanghai Institute of Materia Medica* of *CAS* for recording the HR-ESI-MS.

Experimental Part

General. TLC: Silica gel $60F_{254}$ (SiO_2; *Qingdao Haiyang Chemical Co., Ltd.*, P. R. China), visualization by spraying with 10% H_2SO_4 and heating. Column chromatography (CC): *Diaion HP-20* (*Mitsubishi Kasei*, Japan) and *ODS* (40–63 mm, *Merck*, USA). Prep. HPLC: *ODS* column (*C-18*, 250 × 20 mm, *Shimadzu Pak*; Detector: RID); flow rate, 10 ml/min. M.p.: *Yanaco MP-S3* micromelting point apparatus; uncorrected. Optical rotations: *P-1020* digital polarimeter (*Jasco Corporation*, Japan). ^{1}H- and ^{13}C-NMR, along with 2D-NMR spectra: *Bruker AV-400* (at 400 (^{1}H) and 100 MHz (^{13}C)) spectrometer; TMS as an internal standard; chemical shifts (δ) in ppm and coupling constants (J) in Hz. ESI-MS: *Bruker esquire 2000* mass spectrometer.

Plant Material. The roots of *Ardisia crenata* SIMS were collected from Guangxi Province of China in 2000, and identified by Prof. *Qishi Sun*. A voucher specimen (YL-2001-113) has been deposited with the Shenyang Pharmaceutical University, Liaoning Province of China.

Extraction and Isolation. The air-dried roots of *Ardisia crenata* SIMS (7 kg) were extracted with 60% EtOH (2 × 10 l) for 2 h under reflux to give 500 g of crude extract. A portion of the crude extract (200 g) was then suspended in H_2O, and subsequently partitioned with AcOEt and BuOH. The BuOH extract was subjected to *Diaion HP-20* chromatography eluted stepwise with H_2O, 30% EtOH, 50% EtOH, 70% EtOH, and 95% EtOH to afford seven fractions, *Frs. ACB-1–ACB-7*). *Fr. ACB-5* (20 g) was separated by an *ODS* open column with H_2O/MeOH gradient to yield six subfractions, *Frs. ACB-51–ACB-56*. *Fr. ACB-54* (2.6 g), eluted with 50% MeOH, was further separated by prep. HPLC with MeOH/H_2O 3:2 to give eight subfractions, *Frs. ACB-541–ACB-548*. The purification of *Fr. ACB-543* (40 mg) on prep. HPLC with MeOH/H_2O 2:3 yielded 8 mg of compound **8**. Prep. HPLC of *Fr. ACB-544* (56 mg) with MeOH/H_2O 2:3 afforded 10 mg of compound **9**. By the same method, *Frs. ACB-545* (310 mg) and *ACB-546* (440 mg) were purified by prep. HPLC with MeOH/H_2O 1:1 to furnish **6** (135 mg) and **7** (160 mg). *ODS* Chromatography of a portion of *Fr. ACB-55* (1 g) with MeOH/H_2O 3:2, followed by crystallization from MeOH, afforded compounds **4** (520 mg) and **5** (165 mg). *Fr. ACB-6* (7 g) was submitted to CC (*ODS*; 60% MeOH) and further purified by prep. HPLC (55% MeOH) to afford compounds **1** (90 mg), **2** (65 mg), and **3** (15 mg). *Fr. ACB-7* (11 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH of increasing polarity) to give twelve fractions, *Frs. ACB-71–ACB-712*. *Fr. ACB-711* was further separated by prep. HPLC (MeOH/H_2O 7:3) to afford compounds **10** (22 mg) and **11** (34 mg).

Ardisicrenoside I (*=(3β,16α)-16-Hydroxy-30,30-dimethoxy-13,28-epoxyoleanan-3-yl β-*D*-Glucopyranosyl-(1 → 2)-[β-*D*-xylopyranosyl-(1 → 2)-β-*D*-glucopyranosyl-(1 → 4)]-α-*L*-arabinopyranoside*; **1**). White amorphous powder (MeOH). M.p. 289°. $[\alpha]_D^{25} = +12.2$ ($c = 0.117$, MeOH). ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS (pos.): 1129. ESI-MS (neg.): 1105. HR-ESI-MS (pos.): 1129.5822 ($C_{54}H_{90}NaO_{23}^+$; calc. 1129.5771).

Ardisicrenoside J (*=(3β,16α)-16-Hydroxy-30,30-dimethoxy-13,28-epoxyoleanan-3-yl β-*D*-Glucopyranosyl-(1 → 2)-[α-*L*-rhamnopyranosyl-(1 → 2)-β-*D*-glucopyranosyl-(1 → 4)]-α-*L*-arabinopyranoside*; **2**). White amorphous powder (MeOH). M.p. 294–295°. $[\alpha]_D^{25} = -15.7$ ($c = 0.124$, MeOH). ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS (pos.): 1143. ESI-MS (neg.): 1119. HR-ESI-MS (pos.): 1143.5934 ($C_{55}H_{92}NaO_{23}^+$; calc. 1143.5927).

Ardisicrenoside M (*=(3β)-30,30-Dimethoxy-16-oxo-13,28-epoxyoleanan-3-yl β-*D*-Glucopyranosyl-(1 → 2)-[β-*D*-xylopyranosyl-(1 → 2)-β-*D*-glucopyranosyl-(1 → 4)]-α-*L*-arabinopyranoside*; **3**). White amorphous powder (MeOH). M.p. 271–272°. $[\alpha]_D^{25} = -34.6$ ($c = 0.102$, MeOH). ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS (pos.): 1127. ESI-MS (neg.): 1105. HR-ESI-MS (pos.): 1127.5682 ($C_{54}H_{88}NaO_{23}^+$; calc. 1127.5614).

Acid Hydrolysis. Compound **2** (*ca.* 2 mg) was heated in 1M HCl at 80° for 10 h. The mixture was then neutralized with 1M NaOH and filtered. The filtrate was partitioned with $CHCl_3$. The H_2O layer was concentrated, and rhamnose, glucose, and arabinose were identified by TLC comparison with authentic samples. Compounds **1** (2 mg) and **3** (*ca.* 2 mg) were treated in the same way, and xylose, glucose, and arabinose were identified by TLC analysis.

Cytotoxicity Assay. Cancer cells were cultured in PRMI-1640 medium supplemented with 5% fetal bovine serum. The cultures were incubated at 37° in a 5% CO_2 humidified incubator and subcultured every 2 d to maintain them in a state of logarithmic growth. Then, the cells were seeded into 96-well microtiter plates (1×10^4 cells per well). Compounds were dissolved in DMSO and added to the 96-well microtiter plates 24 h after seeding. The cells were incubated for 2 d in the presence of sample. For the evaluation of *in vitro* cytotoxicity, MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay was used. The anticancer drug *cis*-dichlorodiamine platinum (*cis*-DDP) was used as the pos. control.

REFERENCES

[1] M. Vermeersch, K. Foubert, R. I. da Luz, L. Van Puyvelde, L. Pieters, P. Cos, L. Maes, *Phytother Res.* **2009**, *23*, 1404.
[2] D.-L. Liu, X.-M. Zhang, N.-L. Wang, X.-S. Yao, *J. Shenyang Pharm. Univ.* **2004**, *21*, 394.
[3] Y. Tian, H.-F. Tang, F. Qiu, X.-J. Wang, X.-L. Chen, A.-D. Wen, *Planta Med.* **2009**, *75*, 70.
[4] H.-F. Tang, J. Yun, H.-W. Lin, X.-L. Chen, X.-J. Wang, G. Cheng, *Chem. Biodiversity* **2009**, *6*, 1443.
[5] P.-C. Zhang, C.-F. Zheng, C.-S. Wu, Y.-X. Sheng, J.-L. Zhang, *J. Asian Nat. Prod. Res.* **2010**, *12*, 64.
[6] P. Wen, X.-M. Zhang, Z. Yang, N.-L. Wang X.-S. Yao, *J. Asian Nat. Prod. Res.* **2008**, *10*, 873.
[7] D.-L. Liu, N.-L. Wang, X. Zhang, H. Gao, X.-S. Yao, *J. Asian Nat. Prod. Res.* **2007**, *9*, 119.
[8] Jiangsu New Medical College, 'Dictionary of Chinese Traditional Medicine', Shanghai Science and Technology Press, 1986, pp. 2358 – 2360.
[9] C. Jansakul, H. Baumann, L. Kenne, G. Samuelsson, *Planta Med.* **1987**, *53*, 405.
[10] P.-K. Agrawal, *Phytochemistry* **1992**, *31*, 3307.

Received July 29, 2010