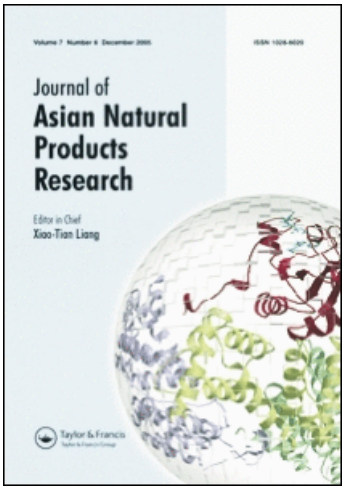

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Two new triterpenoid saponins from *Ardisia crenata*

D. -L. Liu[a]; N. -L. Wang[ab]; X. Zhang[a]; H. Gao[a]; X. -S. Yao[ab]
[a] Department of Natural Products Chemistry, Shenyang Pharmaceutical University, Shenyang, China [b] Shenzhen Research Center of Traditional Chinese Medicines & Natural Products, Shenzhen, China

To cite this Article Liu, D. -L. , Wang, N. -L. , Zhang, X. , Gao, H. and Yao, X. -S.(2007) 'Two new triterpenoid saponins from *Ardisia crenata*', Journal of Asian Natural Products Research, 9: 2, 119 — 127
To link to this Article: DOI: 10.1080/10286020412331286443
URL: http://dx.doi.org/10.1080/10286020412331286443

Journal of Asian Natural Products Research, Vol. 9, No. 2, March 2007, 119–127

Two new triterpenoid saponins from *Ardisia crenata*

D.-L. LIU†, N.-L. WANG†‡, X. ZHANG†, H. GAO† and X.-S. YAO†‡*

†Department of Natural Products Chemistry, Shenyang Pharmaceutical University, Shenyang, 110016, China
‡Shenzhen Research Center of Traditional Chinese Medicines & Natural Products, Shenzhen, 518057, China

(Received 18 February 2004; revised 20 April 2004; in final form 28 April 2004)

Two new triterpenoid saponins, ardisicrenoside K (**1**) and ardisicrenoside L (**2**), have been isolated from the roots of *Ardisia crenata* Sims. Their structures have been determined as 3β-*O*-{α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 4)-[β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranosyl}-13β,28-epoxy-16-oxo-30, 30-dimethoxyoleanane and 3β-*O*-{β-D-xylopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 4)-[β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranosyl}-13β,28-epoxy-16α,20-dihydroxyoleanane by means of chemical evidences and spectral analysis. Their weak anti-fungal activity against the plant pathogenic fungus *Pyricularia oryzae* was evaluated *in vitro*.

Keywords: *Ardisia crenata* Sims; Triterpenoid saponin; Ardisicrenoside K; Ardisicrenoside L; Anti-fungal

1. Introduction

Previous chemical studies have revealed triterpenoid saponins as the main components of plants of the genus *Ardisia* (Myrsinaceae) [1,2], which have been traditionally used for the treatment of activating blood to eliminate stasis. Recently, more and more structurally novel triterpenoid saponins have been isolated from this genus *Ardisia* [3–10]. They exhibit a wide range of bioactivities: utero-contracting, inhibitory activity on cAMP phosphodiesterase, cytotoxicity, anti-HIV and anti-cancer *etc.* [2].

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 [11]. It is a shrub widely distributed in the south of China. The ethanol extract of *Ardisia crenata* Sims also showed good activity against the growth of *Pyricularia oryzae in vitro*, a bioassay system for detecting anti-cancer agents in our preliminary screening. Triterpenoid saponins were detected as the main components in the ethanol extract of *A. crenata* by TLC and color reactions.

*Corresponding author Address: Room 315, L Block, Graduate School at Shenzhen, Tsinghua University, University Town of Shenzhen, Shenzhen, China. Tel.: +86-755-26036137. Fax: +86-755-26036131. Email: yaoxinsheng@hotmail.com; Email: yaoxinsheng@163.net

Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online 2007 © Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020412331286443

We report here the isolation and structural elucidation of two new triterpenoid saponins, ardisicrenoside K and ardisicrenoside L, obtained from the roots of *A. crenata* Sims, as well as their anti-fungal activity against *Pyricularia oryzae*.

2. Results and discussion

The roots of *A. crenata* Sims were extracted with 60% ethanol. The ethanol extract was then dissolved in water and subsequently partitioned with ethyl acetate and n-butanol. The n-butanol-soluble portion was subjected to column chromatography on Diaion HP-20, MPLC RP-18. The saponin-containing fractions were further chromatographed, by repeated HPLC on an ODS column, to yield two new triterpenoid saponins, ardisicrenoside K (**1**) and ardisicrenoside L (**2**).

Ardisicrenoside K (**1**), white needles, mp 248–250°C gave a positive Liebermann–Burchard reaction and Molish test. The positive HR-ESIMS spectrum revealed a quasi-molecular ion peak at *m/z* 1141.5802 $[M + Na]^+$ corresponding to a molecular formula of $C_{55}H_{90}O_{23}$. The IR spectrum of compound **1** exhibits a broad absorption of hydroxyl group at 3417 cm^{-1}, and carbonyl absorption at 1701 cm^{-1}. The ^1H NMR spectrum of compound **1** in pyridine-d_5 exhibits signals of six tertiary methyl groups at δ 1.17, 1.30, 1.09, 1.07, 1.04 and 0.83, a methyl group of rhamnose at δ 1.80 (d, $J = 6.0$ Hz), two methoxyl groups at δ 3.49 and 3.48, and four anomeric proton signals from sugars at δ 6.40 (brs), 5.38 (d, $J = 7.8$ Hz), 5.26 (d, $J = 7.6$ Hz) and 4.95 (brs). The above data suggest that ardisicrenoside K contains four sugars moieties and a triterpenoid aglycone. Complete analysis of the ^{13}C NMR and DEPT spectra of compound **1** revealed 55 carbon signals, 32 of which were assigned to the aglycone part, while 23 were assigned to the sugar moiety. ^{13}C NMR spectral data of compound **1** are similar to those of the known saponin ardisimamilloside B [4]. As shown in table 1, there is a signal at δ 107.9 (CH, by DEPT) instead of a signal at δ 206.2 due to the 30-CHO group of ardisimamilloside B. Furthermore, in the HMBC spectrum of compound **1**, the carbon signal at δ 107.9 not only correlates with Me-29, but also with two methoxyl signals at δ 3.49 (3H, *s*) and 3.48 (3H, *s*), confirming that δ 107.9 is the signal of C-30 and the two methoxyl groups are at C-30 (figure 1). The hydroxyl group at C-3 for the aglycone was deduced from the signal at δ 89.1 and its configuration was determined by the NOESY spectrum. The NOE correlations of Hax-3 with H-23 (Me) and H-5 indicate a β-configuration for the 3-OH in the aglycone. From the above evidences, the structure of the new sapogenin of compound **1** is established as 3β-hydroxy-16-oxo-13β,28-epoxy-30,30-dimethoxyoleanane.

On acid hydrolysis of **1**, arabinose, glucose and rhamnose were identified by co-TLC with authentic samples. The positive-ion ESIMS spectrum exhibits a single predominant peak at *m/z* 1141, assigned to $[M + Na]^+$. It afforded peaks at *m/z* 995 $[M + Na - 146]^+$ and 833 $[M + Na - 146 - 162]^+$ in its $ESIMS^2$ spectrum. The peak at *m/z* 833 gave a prominent ion at *m/z* 671 $[M + Na - 146 - 162 \times 2]^+$ in the $ESIMS^3$ spectrum. In combination with the ESIMS results, it can be concluded that the ratio of arabinose, glucose and rhamnose in **1** is 1:2:1. ^1H—^1H COSY, TOCSY, HMBC and NOESY spectral analysis were used to determine the sequence of the oligosaccharide chain in **1**. From the relatively large H-1 coupling constants (7.8, 7.6 Hz), the anomeric hydroxyl of both glucose moieties should be β-configuration [5]. The small H-1 coupling constants of arabinose and rhamnose, which exhibit a broad anomeric proton singlet in their ^1H NMR spectrum, indicate that they should

Table 1. ^{1}H and ^{13}C NMR data for **1** in C_5D_5N (δ values)[a].

No.	^{13}C	^{1}H	*Ardisimamilloside B*	*No.*	^{13}C	^{1}H
1	39.0 t	1.77 (o), 0.89 (o)[b]	39.0	3-*O*-sugar		
2	26.5 t	1.87 (o), 2.04 (o)	26.5	Ara-1	104.4 d	4.95 (br s)
3	88.9 d	3.15 (dd, $J = 11.6, 4.0$)[c]	89.0	2	80.8 d	4.57 (o)
4	39.6 s		39.6	3	72.4 d	4.47 (o)
5	55.4 d	0.63 (d, $J = 8.3$)	55.5	4	74.6 d	4.58 (o)
6	17.7 t	1.48 (o), 1.36 (o)	17.8	5	63.4 t	4.37 (o), 3.76 (o)
7	33.7 t	1.34 (o), 0.99 (o)	33.8	Glc (terminal)-1	105.4 d	5.38 (d, $J = 7.8$)
8	42.9 s		43.0	2	76.3 d	4.08 (o)
9	50.0 d	1.12 (m)	50.1	3	78.0 d	4.21 (o)
10	36.7 s		36.7	4	71.7 d	4.22 (o)
11	18.8 t	1.48 (o), 1.29 (o)	18.8	5	78.0 d	4.06 (o)
12	31.9 t	2.01 (o), 1.66 (o)	31.6	6	62.9 t	4.39 (o), 4.50 (o)
13	86.3 s		86.2	Glc (inner)-1	103.0 d	5.26 (d, $J = 7.6$)
14	49.8 s		47.9	2	77.2 d	4.28 (o)
15	45.7 t	2.85 (d, $J = 16.1$), 2.02 (m)	45.7	3	79.5 d	4.17 (o)
16	212.6 s		212.6	4	71.8 d	4.13 (o)
17	55.9 s		55.3	5	78.3 d	3.79 (o)
18	53.9 d	2.19 (d, $J = 14.6$)	55.9	6	62.6 t	4.44 (o), 4.28 (o)
19	35.8 t	2.13 (d, $J = 12.4$), 1.38 (o)		Rham-1	101.5 d	6.40 (br s)
20	40.6 s		50.1	2	72.3 d	4.72 (o)
21	31.5 t	1.94 (o), 1.80 (o)	29.6	3	72.7 d	4.67 (dd, $J = 9.2, 3.4$)
22	24.9 t	2.35 (d, $J = 13.2$), 1.40 (o)	33.8	4	74.8 d	4.27 (o)
23	27.9 q	1.17 (s)	28.0	5	69.3 t	5.06 (o)
24	16.4 q	1.04 (s)	16.4	6	18.9 q	1.80 (d, $J = 6.0$)
25	16.0 q	0.83 (s)	16.1			
26	18.7 q	1.30 (s)	18.8			
27	21.7 q	1.09 (s)	21.9			
28	74.9 t	3.94 (d, $J = 8.2$), 3.59 (m)	74.9			
29	23.9 q	1.07 (s)	23.9			
30	107.9 d	4.50 (s)	206.2			
30 OCH_3	58.4 q	3.49 (s)				
30 OCH_3	58.3 q	3.48 (s)				

[a] Recorded on a Bruker AV 400 (400 MHz for ^{1}H, 100 MHz for ^{13}C) NMR spectrometer.
[b] Overlapped signals indicated by (o).
[c] *J*values (in parentheses) are in Hz. The carbon and proton signals were unambiguously assigned through HMQC, HMBC and TOCSY.

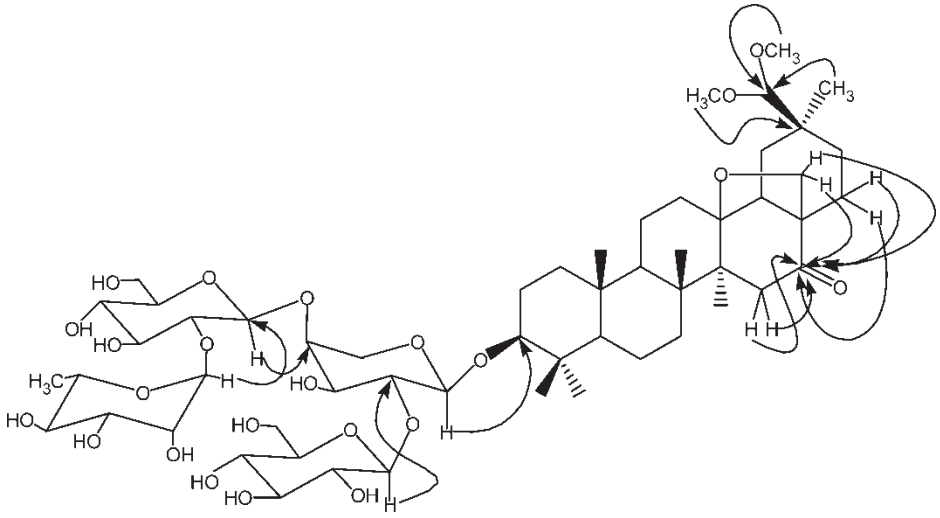

Figure 1. Main HMBC correlations for **1**.

each possess an α-configuration. Based on these results, and by comparison of the signals from the sugars moieties of **1** in the ^{13}C NMR spectrum with the literature [4–6], 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 by comparing the chemical shifts of the individual sugar residues with published compounds [12], and confirmed by HMBC and NOESY experiments. The C-1 of arabinose is attached to C-3 of the aglycone, as indicated by the correlation between H-1 (δ 4.95) of arabinose with C-3 (δ 88.9) of the aglycone in the HMBC spectrum, and between H-1 of arabinose and H-3 (δ 3.15) of the aglycone in the NOESY spectrum. An HMBC experiment of compound **1** revealed the following correlations: H-1 (δ 5.38) of the terminal glucose with C-2 (δ 80.8) of arabinose; H-1 (δ 5.26) of the inner glucose with C-4 (δ 74.6) of arabinose; and H-1 (δ 6.42) of rhamnose with C-2 (δ 77.2) of the inner glucose. On the basis of the above analysis, compound **1** was identified as 3β-*O*-{α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 4)-[β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranosyl}-16-oxo-13β,28-epoxy-30,30-dimethoxyoleanane, named ardisicrenoside K (figure 2).

Ardisicrenoside L (**2**), obtained as a white powder, was positive to both the Liebermann–Burchard reaction and the Molish test. The molecular formula $C_{51}H_{84}O_{22}$ was established by analysis of positive HR-ESIMS, which gave a quasi-molecular ion peak at *m/z* 1071.5396 $[M + Na]^+$. The IR spectrum shows a strong absorption at 3421 cm^{-1} due to hydroxyl groups and a C—O stretching band at 1041 cm^{-1}.

The ^1H NMR spectrum of compound **2** in pyridine-d_5 displays signals for six tertiary methyl groups at δ 1.58, 1.54, 1.36, 1.21, 1.08 and 0.85, a pair of geminal proton signals at δ 3.43 (*s*), 3.64 (*d*, $J = 7.41$) and four anomeric proton signals at δ 5.47 (*d*, $J = 7.6$ Hz), and 4.79 (*d*, $J = 5.9$ Hz). The above data suggest that **2** contains four sugar moieties and a triterpenoid aglycone. The ^{13}C NMR spectrum exhibits 51 carbon signals, 29 of them assigned to the aglycone part and 22 to the sugar moiety. The ^{13}C NMR spectral data of **2** are similar to those of the known saponin ardisicrenoside A [6]. As shown in table 2,

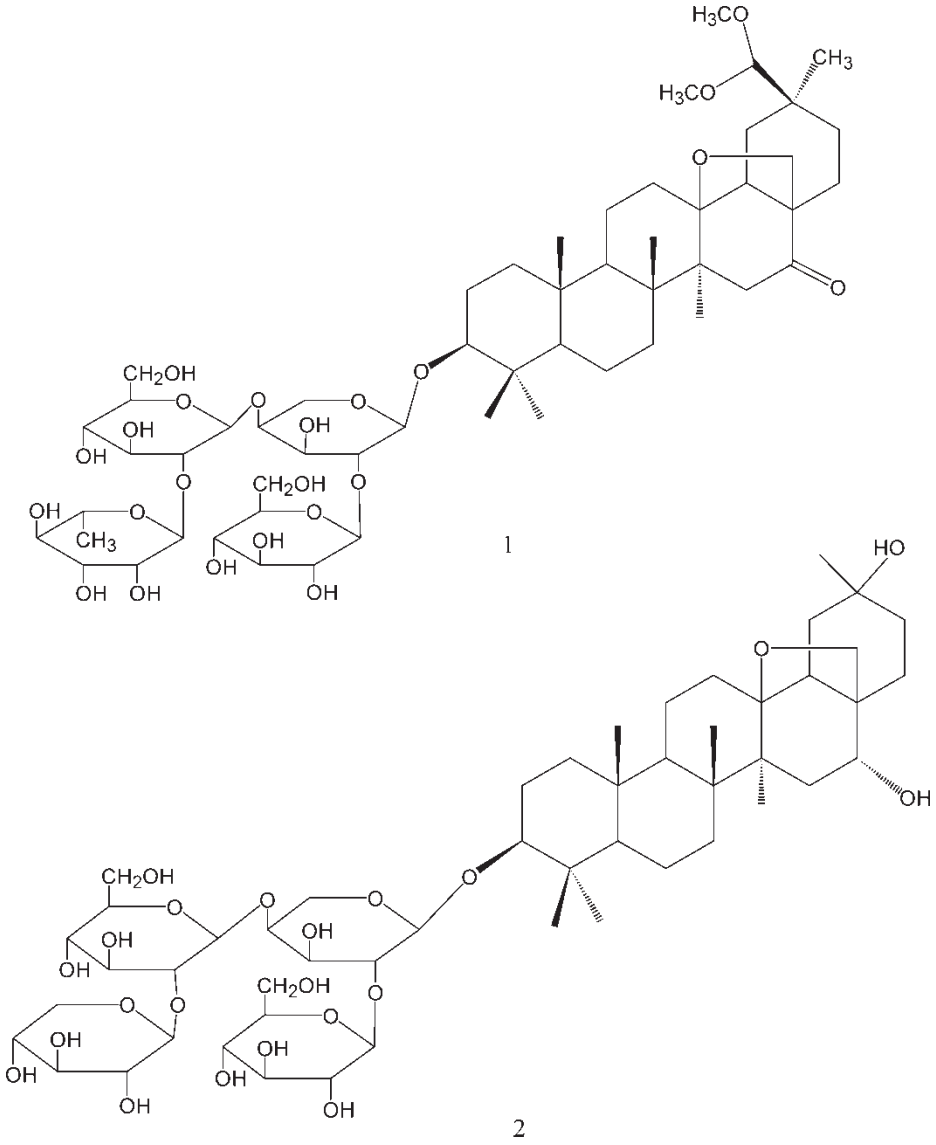

Figure 2. Structures of compounds **1** and **2**.

a signal at δ 69.7 arising from an oxygenated carbon was observed in the ^{13}C NMR of compound **2**, instead of the signals due to the formyl group at C-30 and the quaternary carbon at C-20 of ardisicrenoside A. The existence of the hydroxyl group at C-20 was deduced by the downfield shift at C-19, C-21 and C-29. The oxygenated groups at C-3 and C-16 in the aglycone were deduced from the signals at δ 89.1 and 77.3, respectively. The configuration of oxygenated bond at C-3 and hydroxyl group at C-16 was determined using a NOESY experiment. NOE correlations of Hax-3 (δ 3.17) with H-23 (δ 1.21, Me) and H-5 (δ 0.70), and H-16 (δ 4.28) with H-28 (δ 3.94), indicate a β-configuration for the oxygenated bond at C-3 and α-configuration for 16-OH, respectively. The orientation of 16α-OH was determined by comparing the chemical shift of C-16 (δ 77.3) with that in the

Table 2. ^{1}H and ^{13}C NMR data for compound 2 in C_5D_5N (δ values)[a].

No.	^{13}C	^{1}H	*Ardisiacripin A*	*No.*	^{13}C	^{1}H	*Sugars of ardisiacripin A*
1	39.2 t	1.65 (o), 0.75 (o)[b]	38.9 t	3-*O*-sugar			
2	26.5 t	2.00 (o), 1.86 (o)	26.2 t	Ara-1	104.6 d	4.79 (d, $J = 5.9$)	104.3 d
3	89.1 d	3.17 (o)	88.8 d	2	79.7 d	4.57 (o)	79.3 d
4	39.7 s		39.4 s	3	73.2 d	4.25 (o)	72.7 d
5	55.7 d	0.70 (d, $J = 11.0$)[c]	55.4 d	4	78.5 d	4.23 (o)	78.2 d
6	17.9 t	1.42 (o)	17.6 t	5	64.1 t	4.46 (o), 3.68 (o)	63.8 t
7	37.0 t	2.28 (o), 1.51 (o)	33.9 t	Glc (terminal)-1	104.9 d	5.47 (d, $J = 7.6$)	104.4 d
8	44.7 s		42.2 s	2	76.1 d	4.02 (o)	75.7 d
9	50.5 d	1.33 (o)	50.1 d	3	77.8 d	4.01 (o)	77.6 d
10	36.9 s		36.5 s	4	71.8 d	4.25 (o)	71.3 d
11	19.3 t	1.80 (o), 1.64 (o)	18.8 t	5	77.6 d	4.21 (o)	77.5 d
12	32.8 t	2.15 (o), 1.64 (o)	32.3 t	6	62.9 t	4.55 (o), 4.40 (o)	62.4 t
13	86.4 s		86.1 s	Glc (inner)-1	104.1 d	4.99 (d, $J = 7.9$)	103.8 d
14	42.4 s		44.2 s	2	85.4 d	3.92 (o)	84.9 d
15	34.5 t	1.57 (o), 1.30 (o)	36.3 t	3	77.3 d	4.28 (o)	77.0 d
16	77.3 d	4.28 (o)	76.5 d	4	71.1 d	4.22 (o)	70.5 d
17	44.3 s		43.6 s	5	78.2 d	4.23 (o)	77.8 d
18	50.0 d	2.54 (o)	52.9 d	6	62.3 t	4.43 (o), 4.40 (o)	61.8 t
19	39.5 t	3.00(d, $J = 13.9$), 1.98 (o)	33.0 t	Xyl-1	107.6 d	4.97 (d, $J = 7.3$)	107.2 d
20	69.7 s		47.9 s	2	76.2 d	4.05 (o)	75.6 d
21	37.4 t	2.81 (d, $J = 13.9$), 2.54 (o)	30.1 t	3	77.3 d	4.28 (o)	77.2 d
22	31.4 t	2.12 (m), 1.99 (o)	31.9 t	4	70.7 d	4.12 (o)	70.2 d
23	28.0 q	1.21 (s)	27.7 q	5	67.4 t	4.56 (o), 3.72 (o)	67.0 d
24	16.6 q	1.08 (s)	16.2 q				
25	16.4 q	0.85 (s)	15.9 q				
26	18.6 q	1.36 (s)	18.1 q				
27	19.6 q	1.58 (s)	19.4 q				
28	77.9 t	3.64(s), 3.43 (d, $J = 7.4$)	77.3 t				
29	32.8 q	1.54 (s)	23.7 q				
30			207.3 d				

[a] Recorded on a Bruker AV 400 (400 MHz for ^{1}H, 100 MHz for ^{13}C) NMR spectrometer.
[b] (o) indicates overlapped signals.
[c] J values (in parentheses) are in Hz. The carbon and proton signals were unambiguously assigned through HMQC, HMBC and TOCSY.

literature (16α-OH, δ 77.0; 16β-OH, δ 64.0) [5]. From the above evidences, the structure of the new sapogenin was established as 3β,16α,20-trihydroxy-13β,28-epoxyoleanane.

The ESIMS spectrum of **2** exhibits a single predominant peak at *m/z* 1071, assigned to $[M + Na]^+$, which gave ions at *m/z* 939 $[M + Na - 132]^+$ and 909 $[M + Na - 162]^+$ in its positive-ion $ESIMS^2$ spectrum. The ion peaks at *m/z* 939 and *m/z* 909 gave the same ion at *m/z* 777 $[M + Na - 132 - 162]^+$ in the MS^3 spectrum. The ion peaks at *m/z* 1047 $[M - H]^-$ gave ions at *m/z* 915 $[M - H - 132]^-$ and 735$[M - H - 132 - 162]^-$ in the negative-ion $ESIMS^2$ spectrum, and the MS^3 spectrum of *m/z* 735 gave a prominent ion at *m/z* 591$[M - H - 132 - 162 \times 2]^-$. On acid hydrolysis, **2** afforded arabinose, glucose and xylose in a ratio of 1:2:1 (analyzed by the same method as **1**). By comparing the vicinal coupling constant of anomeric protons with those of published model compounds [5–7], the three sugars were determined to be α-L-arabinopyranose, β-D-glucopyranose and β-D-xylopyranose. Using the same methods as **1**, the C-1 of arabinose was attached to the C-3 of the aglycone. Long-range coupling correlations were observed between H-1 of xylose and C-2 of the inner glucose, H-1 of the inner glucose and C-4 of arabinose, and H-1 of the terminal glucose and C-2 of the arabinose in the HMBC spectrum (figure 3). Based on the above evidences, the structure of **2** was elucidated as 3β-*O*-{β-D-xylopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 4)-[β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranosyl}-13β,28-epoxy-16α,20-dihydroxyoleanane, named ardisicrenoside L (figure 2).

Ardisicrenosides K and L exhibited weak activity against the plant pathogenic fungus *Pyricularia oryzae* with MMDCs (minimal morphological deformation concentration) of 295 and 320 μM, respectively.

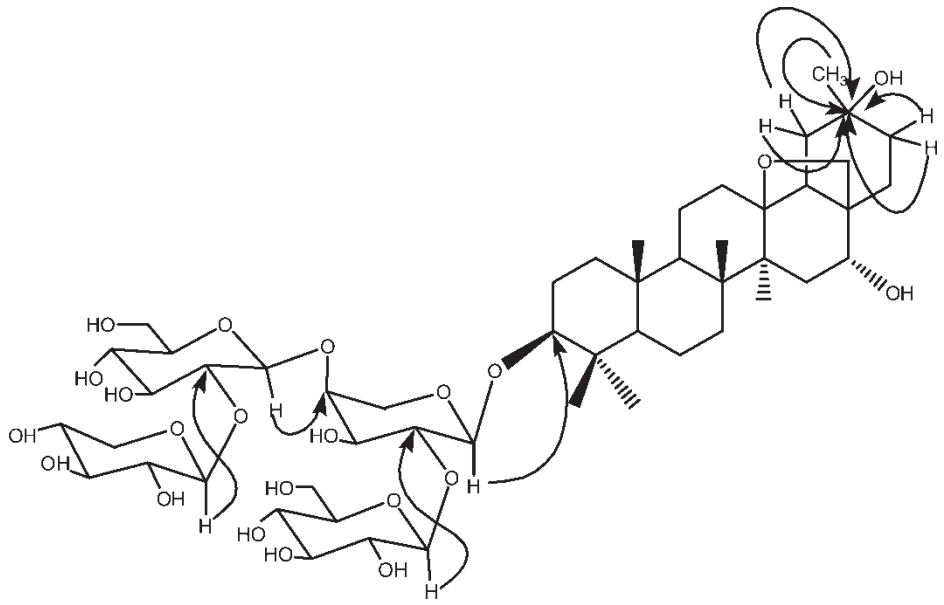

Figure 3. Main HMBC correlations for **2**.

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Yanaco MP-S_3 micromelting point apparatus and are uncorrected. Optical rotations were measured using a P-1020 digital polarimeter (Jasco corporation). ESIMS spectra were obtained using a Bruker esquire 2000 mass spectrometer. IR spectra were recorded on a Shimadzu FT/IR-8400 spectrometer. ^{1}H and ^{13}C NMR, along with 2D NMR spectra, were determined on a Bruker AV-400 (400 MHz for ^{1}H, 100 MHz for ^{13}C) NMR spectrometer, using TMS as an internal standard. Chemical shifts are in δ (ppm) and coupling constants (J) are 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. Diaion HP-20 (Mitsubishi Kasei) and ODS (40–63 μm, Merck) were used for column chromatography. Preparative HPLC was performed using an ODS column (C-18, 250 × 20 mm, Shimadzu Pak; Detector: RID).

3.2 Plant material

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

3.3 Extraction and isolation

The air-dried roots of *Ardisia crenata* Sims (7 kg) were extracted with 60% EtOH (10 l × 2) under reflux. The EtOH extract (500 g) was then suspended in water and subsequently partitioned with ethyl acetate and n-butanol. The n-butanol extract (180 g) was subjected to Diaion HP-20 chromatography using a gradient from H_2O to 95% EtOH. The fraction eluted with 50% EtOH (49 g) was separated by an ODS open column with H_2O-MeOH gradient elution to yield six fractions (1–6). Fraction 4 (2.6 g) was further separated by PHPLC with MeOH–H_2O (3:2) to obtain eight subfractions (41–48). Subfraction 48 (98 mg) was finally purified by PHPLC with MeOH–H_2O (7:3) to afford ardisicrenoside K (15 mg). Ardisicrenoside L (10 mg) was obtained from subfraction 42 (90 mg) by repeated PHPLC with MeOH–H_2O (1:1).

3.3.1 Ardisicrenoside K (1). White needles, mp 248–250°C, $[\alpha]_D^{20}$ −17.5 (c = 0.1, MeOH). IR ν_{max}^{KBr} (cm^{-1}): 3417, 2927, 2869, 1701, 1072, 1045, 891. Positive HR-ESIMS (m/z): $[M + Na]^+$ 1141.5802 (calcd. for $C_{55}H_{90}O_{23}Na$, 1141.5771). Positive ESIMS (m/z): 1141, 995, 979, 833, 777, 643, 625, 497. Negative ESIMS (m/z): 1153, 1117, 1077, 971, 809, 647, 616. ^{1}H and ^{13}C NMR: see table 1.

3.4 Ardisicrenoside L (2)

White powder, $[\alpha]_D^{20}$ −11.4 (c = 0.1, MeOH). IR ν_{max}^{KBr} (cm^{-1}): 3421, 2933, 2873, 1079, 1041, 887. Positive HR-ESIMS (m/z): $[M + Na]^+$ 1071.5396 (calcd. for $C_{51}H_{84}O_{22}Na$, 1071.5352). Positive ESIMS (m/z): 1071, 939, 909, 777, 615. Negative ESIMS (m/z): 1047, 915, 753, 591. ^{1}H and ^{13}C NMR: see table 2.

3.5 Acid Hydrolysis of Saponins

Ardisicrenoside K (2 mg) was heated with 6% HCl at 80°C for 10 h. The reaction mixture was then neutralized with 1 M NaOH and filtered. The filtrate was partitioned with $CHCl_3$. The water layer was concentrated and rhamnose, glucose and arabinose were identified by TLC in comparison with authentic samples.

Ardisicrenoside L (2 mg) was treated with the same way, and xylose, glucose and arabinose were identified by TLC analysis.

3.6 Bioassay

Antifungal assays against the plant pathogenic fungus *Pyricularia oryzae* were carried out as previously reported [13].

Acknowledgements

The authors gratefully acknowledge Professor Qishi Sun of Department of Pharmacognosy, Shenyang Pharmaceutical University for identification of the plant. Thanks are also extended to the Shanghai Institute of Materia Medica of CAS for their assistance with HR-ESIMS.

References

[1] Y. Zhao, H.G. Liu. *Zhongcaoyao*, **30**, 228 (1999).
[2] S.L. Su, Y.H. Li, Z. Ouyang. *Zhongyaocai*, **26**, 144 (2003).
[3] M.T. Wang, X.T. Guan, X.W. Han. *Planta Med.*, **58**, 205 (1992).
[4] J. Huang, Y. Ogihara, H. Zhang, N. Shimizu, T. Takeda. *Phytochemistry*, **54**, 817 (2000).
[5] J. Huang, Y. Ogihara, H. Zhang, N. Shimizu, T. Takeda. *Chem. Pharm. Bull.*, **48**, 1413 (2000).
[6] Z.H. Jia, K. Koike, T. Ohmoto, M.Y. Ni. *Phytochemistry*, **37**, 1389 (1994).
[7] Z.H. Jia, K. Koike, T. Nikaido, T. Ohmoto, M.Y. Ni. *Chem. Pharm. Bull.*, **42**, 2309 (1994).
[8] Z.H. Jia, K. Koike, T. Nikaido, T. Ohmoto. *Tetrahedron*, **50**, 11853 (1994).
[9] K. Koike, Z.H. Jia, S. Ohura, S. Mochida, T. Nikaido. *Chem. Pharm. Bull.*, **47**, 434 (1999).
[10] J. Huang, H. Zhang, N. Shimizu, T. Takeda. *Chem. Pharm. Bull.*, **51**, 875 (2003).
[11] Jiangsu New Medical College (1985) *Dictionary of Chinese Traditional Medicine* (Shanghai Science and Technology Press, Shanghai), p. 913.
[12] K. Pawan. *Phytochemistry*, **31**, 3307 (1992).
[13] K. Hu, A.J. Dong, H. Kobayashi, S. Iwasaki. *Planta Med.*, **62**, 573 (1996).