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New triterpenoid sapoin from *Ardisia gigantifolia* Stapf.

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Abstract

One new triterpenoid sapoin with two known triterpenoid sapoins: 3β-*O*-{α-L-rhamnopyranosyl-(1 → 3)-[β-D-xylopyranose-(1 → 2)]-β-D-glucopyranosyl-(1 → 4)-α-L-arabinopyranosyl}-3β-hydroxy-13β,28-epoxy-oleanan-16-oxo-30-al (**1**), 3β-*O*-{α-L-rhamnopyranosyl-(1 → 3)-[(β-D-xylopyranosyl-(1 → 2)]-β-D-galactopyranosyl-(1 → 4)-[(β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranoside}-16α-hydroxy-13,28-epoxy-oleanane (**2**) and cyclamiretin A 3β-*O*-α-L-rhamnopyranosyl-(1 → 3)-[β-D-xylopyranosyl-(1 → 2)]-β-D-glucopyranosyl-(1 → 4)-[β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranoside (**3**) were isolated from *Ardisia gigantifolia* Stapf. Their structures were elucidated by means of ^{1}H and ^{13}C NMR spectroscopic studies, including 2D-NMR technique.

Keywords: *Ardisia gigantifolia* Stapf.; Myrsinaceae; Triterpenoid sapoin

The dried rhizome of *Ardisia gigantifolia* Stapf. is mainly used as Chinese folk medicine in south of China for the treatment of rheumatism, pain of muscles and bones and traumatic injury. It distributes in the provinces of Guangxi, Jiangxi and Fujian in China, which is a kind of evergreen dwarf shrub [1]. Previous chemical studies showed that triterpenoid saponins were the main components from this genus. Thirty-two triterpenoid saponins have been isolated from the rhizomes of *A. gigantifolia* Stapf. over the last 20 years [2]. In this paper, the isolation and characterization of one new triterpenoid sapoin and two known triterpenoid sapoins from 60% ethanol percolation of the dried rhizome of the plant were reported.

1. Experimental

The dry rhizome of *A. gigantifolia* Stapf. was obtained from Guangdong, China in 2007 and was authenticated by Prof. Ping Liu. The voucher specimen has been deposited in traditional Chinese medicine pharmacy of General Hospital of PLA.

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The dried and cut rhizome parts of *A. gigantifolia* (13.5 kg) was percolated with 60% ethanol. The 60% ethanol extract (1.54 kg) was partitioned successively between water and petroleum, ethyl acetate, *n*-butanol, respectively. After removing the solvent, the *n*-butanol layer (600 g) was separated with macroporous resin D101 eluted with EtOH/H_2O (0:100, 30:70, 50:50, 70:30, and 95:5, v/v) gradient to afford five fractions. Fraction 4 (50 g) was subjected to column chromatography on silica gel several times, which gave rise to **2** (0.7450 g) and **3** (10.0 mg). At last, preparative HPLC [MeOH/H_2O (70:30, v/v), flow rate 15.2 mL/min, 200 nm] to give **1** (0.3950 g).

Compound **1** (10 mg) was heated in an ampule with 7 mL of 2 mol/L HCL at 90 °C for 2 h. The aglycone was extracted with EtOAC and H_2O. The H_2O layer was evaporated under reduced pressure. Pyridine (1.5 mL) and 3 mg of $NH_2OH{\cdot}HCL$ were added to residue, and the mixtures were agitated at room temperature for 24 h. Then, Ac_2O (2 mL) was added and mixtures were agitated at room temperature for 24 h. The reaction mixtures were evaporated under reduced pressure, and the resulting aldononitrile peracetates were analyzed by GC-MS using standard aldononitrile peracetates as reference samples.

The GC–MS experiment was carried out on a Shimadzu GC–MS chromatograph using a DB-17 MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film), ion source temperature: 250 °C, interface temperature: 300 °C, and split ratio: 10.0. Helium (1.16 mL/min) was used as carrier gas. The initial column oven temperature was 100 °C for 1 min; then the temperature was increased by 20 °C/min to a value of 200 °C for 1 min; then the temperature was increased by 8 °C/min to a final value of 280 °C. Compound **1** gave L-rhamaose, L-arabinose, D-xylose and D-glucose at t_R 8.693, 8.983, 8.713 and 11.737 min.

2. Results and discussion

The dried and cut rhizome parts of *A. gigantifolia* were percolated with 60% ethanol and the ethanol extract was partitioned between water and petroleum, ethyl acetate and *n*-butanol, respectively. Chromatography of the *n*-butanol extract on silica gel, macroporous resin D101 and Sephadex LH-20, followed by repeated HPLC purification over ODS, afforded compounds **1**–**3**. Compound **1**, obtained as a white powder, showed positive Libermann–Burchard and Molish reactions, suggesting that **1** might be a triterpenoid sapoin or steroidal sapoin. HR-ESI-MS of **1** showed the quasi-molecular ion at *m/z* 1065.5246 $[M+Na]^+$ and 1041.5270 $[M-H]^-$, establishing the molecular formula of

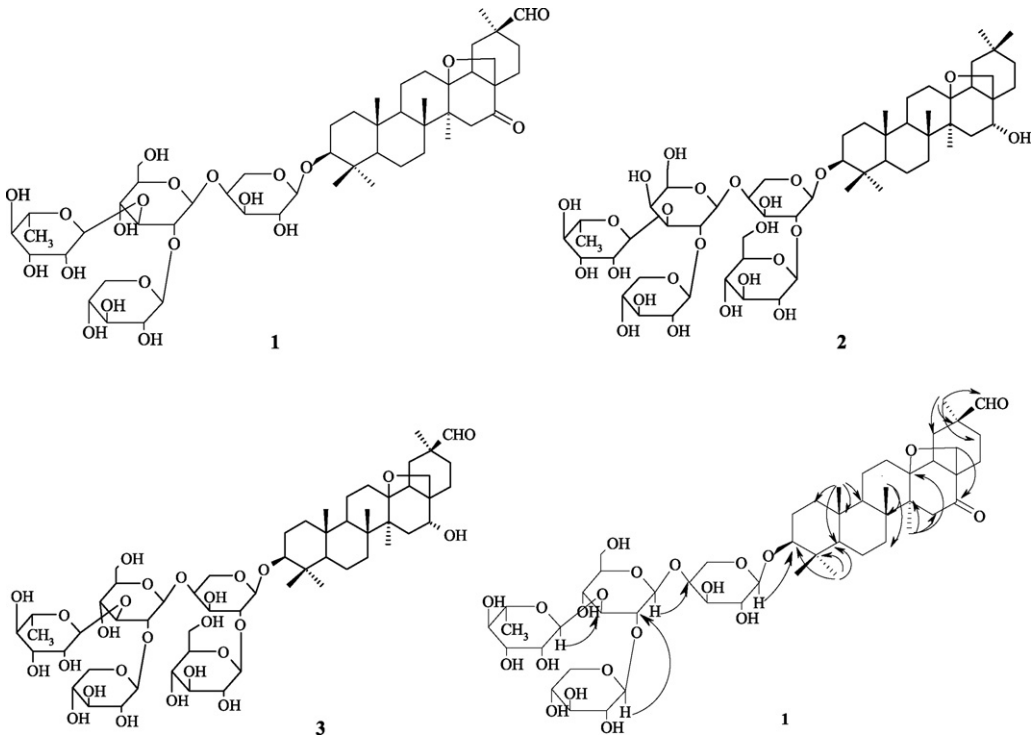

Fig. 1. Structures of compounds **1**–**3** and key HMBC correlations of compound **1**.

$C_{52}H_{82}O_{21}$. The ^{13}C NMR spectral data of the sapogenin part of **1** were similar to those of **3**, which is the known oleanane type triterpene cyclamiretin A. In **3**, the 13β,28-epoxy bridge and 16α-OH is evident from the ^{13}C NMR resonances at δ 86.0 (C-13, C by DEPT), 77.3 (C-28, CH_2) and 76.5 (C-16, C-H), respectively. However, in compound **1**, no resonance was observed for C-16 at δ 76.5; instead, a signal was seen at δ 212.6 (C by DEPT), indicating that the –OH at C-16 of compound **3** is oxidized to a carbonyl group. This assignment was confirmed low-field shifts at C-17 (+11.7), C-15 (+9.2) and C-14 (+5.9), and the high-field shifts at C-28 (−3.0). Furthermore, long-range coupling of H-28 with C-16 in HMBC also supported the same conclusion. Further, in the ^1H NMR spectrum, one carbinylic proton signal assignable to H-3 of the aglycon were observed at δ 3.25(dd, J = 12.0, 4.0 Hz), suggesting the carbinylic protons could be placed at 3α [3]. Based on these findings, the aglycone was identified as 3β-hydrox-13β,28-epoxy-oleanan-16-oxo-30-al (cyclamigenin B).

The monosaccharides obtained after acid hydrolysis of **1** were derivatized into aldononitrile peracetatederivative and analyzed by GC–MS using authentic samples as References L-rhamaose, L-arabinose, D-xylose, and D-glucose in the relative proportions of 1:1:1:1 were detected. The ^1H NMR spectrum showed four anomeric proton signals at δ 4.90 (d, 1H, J = 7.8 Hz, Glc-H-1), 4.91 (d, 1H, J = 8.0 Hz, Xyl-H-1), 4.66 (d, 1H, J = 5.2 Hz, Ara-H-1) and 6.00 (brs, 1H, Rha-H-1). All proton signals due to sugars were assigned by careful analysis of the TOCSY, and HMQC spectra, and the carbon signals were assigned by HMQC and DEPT spectra. The β-anomeric configurations for the glucopyranose and xylopyranose units were determined from their $^3J_{H\text{-}1,\ H\text{-}2}$ coupling constants (6.9–7.8 Hz). The small H-1 coupling constant of arabinose, which indicated the arabinose should have an α-configuration at its anomeric carbon. The broad singlet of the anomeric proton of the Rha unit indicated an α-orientation [4]. The sugar signals of **1** were similar with known **3** [5], **1** was one glucopyranosyl unit less than **3**. The arabinose was connected to C-3 of the aglycon, which was

Table 1
The NMR spectral data of compound **1** (400 MHz, pyridine-d_5).[a,b]

No.	δ_C (DEPT)	δ_H (J_{HZ})	No.	δ_C (DEPT)	δ_H (J_{HZ})
1	39.1 (CH_2)	0.78, 1.62	Arabinose (A)		
2	26.7 (CH_2)	1.84, 2.08	A-1	107.4 (CH)	4.66 d (5.2)
3	88.6 (CH)	3.25 dd (4.0, 12.0)	A-2	73.8 (CH)	4.30
4	39.7 (C)	–	A-3	74.3 (CH)	4.01
5	55.5 (CH)	0.69 d (9.2)	A-4	81.5 (CH)	4.14
6	17.7 (CH_2)	1.42	A-5	65.9 (CH_2)	3.72 d (11.8), 4.64 dd (3.2, 11.8)
7	33.8 (CH_2)	0.94	Glucose (G)		
8	43.0 (C)	–	G-1	105.2 (CH)	4.90 d (7.8)
9	50.0 (CH)	1.06	G-2	81.8 (CH)	4.08
10	36.7 (C)	–	G-3	86.0 (CH)	4.12
11	18.8 (CH_2)	1.37, 1.75	G-4	70.6 (CH)	4.13
12	31.5 (CH_2)	2.05	G-5	78.0 (CH)	3.69
13	86.1(C)	–	G-6	62.1 (CH_2)	4.32, 4.42
14	50.1(C)	–	Xylose(X)		
15	45.7 (CH_2)	1.27, 1.95	X-1	106.0 (CH)	4.91 d (8.0)
16	212.6 (C)	–	X-2	75.3 (CH)	4.03
17	55.3 (C)	–	X-3	78.2 CH)	3.99
18	55.7 (CH)	1.88	X-4	69.6 (CH)	4.10
19	33.8 (CH_2)	1.36, 2.77	X-5	66.8 (CH_2)	3.45 t-like (10.4) 4.20 m
20	47.9 (C)	–	Rhamnose(R)		
21	29.6 (CH_2)	1.95	R-1	103.7 (CH)	6.00 br s
22	33.7 (CH_2)	0.97	R-2	72.3 (CH)	5.04 br s
23	28.0 (Me)	1.26 s	R-3	72.6 (CH)	4.52 dd (9.0, 3.0)
24	16.7 (Me)	1.00 s	R-4	73.7 (CH)	4.32
25	16.1 (Me)	0.82 s	R-5	70.5 (CH)	4.85
26	18.8 (Me)	1.28 s	R-6	18.5 (Me)	1.65 d (6.4)
27	21.8 (Me)	1.10 s			
28	74.3 (CH_2)	3.35 d (8.0), 3.87(8.0)			
29	23.8 (Me)	0.89 s			
30	206.1 (CH)	9.63 s			

[a] Assignments based on TOCSY, HMQC and HMBC experiments.
[b] Overlapped signals are reported without designating multiplicity.

deduced from the HMBC correlation between δ_H 4.66 (d, 1H, 5.2 Hz, Ara-H-1) and δ_C 88.6 (C-3). The sequence of the sugar chain at C-3 was further determined by analysis of the HMBC and HMQC NMR spectra. Thus, HMBC correlations were observed between δ_H 4.90 (Glc-H-1) and δ_C 81.5 (Ara-C-4), δ_H 4.91 (Xyl-H-1) and δ_C 81.8 (Glc-C-2), δ_H 6.00 (Rha-H-1) and δ_C 86.0 (Glc-C-3). On the basis of the above results, the structure of compound **1** was concluded to be 3β-*O*-{α-L-rhamnopyranosyl-(1 → 3)-[β-D-xylopyranose-(1 → 2)]-β-D-glucopyranosyl-(1 → 4)-α-L-arabinopyranosyl}-3β-hydroxy-13β,28-epoxy-oleanan-16-oxo-30-al (Fig. 1 and Table 1).

The structures of the known compounds were identified by spectral data comparison with 3β-*O*-{α-L-rhamnopyranosyl-(1 → 3)-[(β-D-xylopyranosyl-(1 → 2)]-β-D-galactopyranosyl-(1 → 4)-[(β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranoside}-16α-hydroxy-13,28-epoxy-oleanane (**2**) [6] and cyclamiretin A 3β-*O*-α-L-rhamnopyranosyl-(1 → 3)-[β-D-xylopyranosyl-(1 → 2)]-β-D-glucopyranosyl-(1 → 4)-[β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranoside (**3**) [5].

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