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A new triterpenoid and a new glycoside from *Pilea cavaleriei*

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A new triterpenoid, 11α,12α-epoxy-3β-hydroxy-24-nor-olean-4(23)-en-28,13β-olide **(1)**, and a new glycoside, benzyl 2-*O*-β-D-apiofuranosyl-(1 → 2)-β-D-glucopyranosyl benzoate **(2)**, together with eight known triterpenoids (**3**–**10**), were isolated from *Pilea cavaleriei* subsp. *cavaleriei*. Their structures were established on the basis of spectroscopic analysis including HR-ESI-MS, 1D NMR, and 2D NMR techniques. All compounds showed no anti-hepatitis C virus activity.

Keywords: *Pilea cavaleriei* subsp. *cavaleriei*; benzyl 2-*O*-β-D-apiofuranosyl-(1 → 2)-β-D-glucopyranosyl benzoate; 11α,12α-epoxy-3β-hydroxy-24-nor-olean-4(23)-en-28,13β-olide; anti-hepatitis C virus activity

1. Introduction

The genus *Pilea* belongs to the Urticaceae family and widely distributed in tropical and subtropical regions of the world [1]. In China, approximately 30 *Pilea* species are used as traditional Chinese medicines [2]. *Pilea cavaleriei* subsp. *cavaleriei* is a common Zhuang folk medicine and used to treat cough with lung heat, tuberculosis, and traumatic injuries [3]. This paper deals with the isolation and structural elucidation of a new triterpenoid, 11α,12α-epoxy-3β-hydroxy-24-nor-olean-4(23)-en-28,13β-olide **(1)**, and a new glycoside, benzyl 2-*O*-β-D-apiofuranosyl-(1 → 2)-β-D-glucopyranosyl benzoate **(2)**, together with eight known triterpenoids (**3**–**10**). Compounds **3**–**10** were isolated from the genus *Pilea* for the first time. Pentacyclic triterpenoids had been reported possessing anti-hepatitis C virus (anti-HCV) activity [4]. Thus, anti-HCV activity was evaluated and none of the compounds showed anti-HCV activity.

2. Results and discussion

Compound **1** (Figure 1) was obtained as colorless powder. The molecular formula was assigned to be $C_{29}H_{42}O_4$ based on (+)-HR-ESI-TOF-MS data at *m/z* 455.3161 $[M + H]^+$. The ^{1}H NMR spectrum showed signals due to the protons of five tertiary methyl groups at δ 0.84 (3H, s), 0.93 (3H, s), 1.00 (3H, s), 1.10 (3H, s), and 1.14 (3H, s); two exomethylene protons at δ 5.08 (1H, s) and 4.71 (1H, s); and three oxygenated methine protons at δ 4.04 (1H, dd, $J = 10.8$ and 5.6 Hz), 3.06 (1H, br.s), and 3.05 (1H, br.s). Besides, there were 21 overlapping signals at δ 1.19–2.34. The ^{13}C NMR spectrum exhibited 29 signals including 1 carbonyl carbon signal at δ 179.2; 2 exocyclic double bond signals at δ 102.9 and 151.8; 3 oxygenated methine signals at δ 72.9, 57.1, and 52.9; 1 oxygenated

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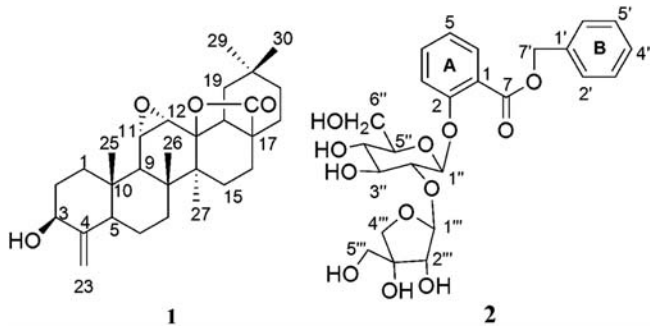

Figure 1. Structures of **1** and **2**.

quaternary carbon signal at δ 87.5; 5 methyl signals at δ 33.2, 23.6, 19.7, 18.8, and 14.3; 3 methine signals at δ 49.7, 49.6, and 48.6; 5 quaternary carbon signals at δ 43.8, 41.3, 40.7, 38.3, and 31.4; and 9 methylene quaternary carbon signals at δ 38.2, 37.8, 34.3, 32.2, 29.6, 27.0, 26.7, 21.3, and 20.3.

^{1}H and ^{13}C NMR spectral data of **1** were assigned based on ^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY, HSQC, and HMBC analyses (Table 1, Figure 2). ^{13}C NMR spectrum exhibited characteristic signals of epoxy-γ-lactones at δ 52.9, 57.1, and 87.5 [5]. ^{1}H–^{1}H COSY spectrum showed that H-23 at δ 5.08 and 4.71 coupled with H-3 at δ 4.04 and H-5 at δ 1.56, respectively. In HMBC spectrum, H-23 at δ 5.08 and 4.71 correlated with C-3 at δ 72.9, C-4 at δ 151.8, and C-5 at δ 49.7; H-3 at δ 4.04 correlated with C-4 at δ 151.8 and C-23 at δ 102.9; H-5 at δ 1.56 correlated with C-3 at δ 72.9, C-4 at δ 151.8, and C-23 at δ 102.9, establishing the presence of an exomethylene group at C-4. ^{1}H–^{1}H COSY spectrum showed H-11 at δ 3.05 coupled with H-9 at δ 1.71, and the HMBC spectrum showed H-11 at δ 3.05 correlated with C-8 at δ 41.3, C-9 at δ 48.6, C-10 at δ 38.3, C-12 at δ 57.1, and C-13 at δ 87.5; H-9 at δ 1.71 correlated with C-8 at δ 41.3, C-10 at δ 38.3, C-11 at δ 52.9, and C-12 at δ 57.1, suggesting the presence of 11,12-epoxy group. HMBC spectrum showed that H-16 at δ 2.15 and 1.33 and H-22 at δ 1.67 correlated with C-28 at δ 179.2 and C-17 at δ 43.8, indicating the presence of 28,13-lactone moiety. HMBC spectrum showed that H-25 at δ 0.84 correlated with C-1 at δ 38.2, C-5 at δ 49.7, C-9 at δ 48.6, and C-10 at δ 38.3; H-26 at δ 1.10 correlated with C-7

Table 1. ^{1}H and ^{13}C NMR spectral data for **1** ($CDCl_3$, TMS, δ).

	1	
Position	δ_C, DEPT	δ_H (*J* in Hz)
1	38.2, CH_2	1.97, m; 1.35, m
2	32.2, CH_2	2.07, m; 1.50, m
3	72.9, CH	4.04, dd (10.8, 5.6)
4	151.8, C	
5	49.7, CH	1.56, d (13.2)
6	20.3, CH_2	1.65, m; 1.49, m
7	29.6, CH_2	1.34, m; 1.19, br.d (12.8)
8	41.3, C	
9	48.6, CH	1.71, m
10	38.3, C	
11	52.9, CH	3.05, br.s
12	57.1, CH	3.06, br.s
13	87.5, C	
14	40.7, C	
15	27.0, CH_2	1.65, m
16	21.3, CH_2	2.15, m; 1.33, m
17	43.8, C	
18	49.6, CH	2.34, dd (13.6,3.2)
19	37.8, CH_2	1.89, t (13.6); 1.62, m
20	31.4, C	
21	34.3, CH_2	1.36, 2H, m
22	26.7, CH_2	1.67, 2H, m
23	102.9, CH_2	5.08, s; 4.71, s
25	14.3, CH_3	0.84, 3H, s
26	19.7, CH_3	1.10, 3H, s
27	18.8, CH_3	1.14, 3H, s
28	179.2, C	
29	23.6, CH_3	0.93, 3H, s
30	33.2, CH_3	1.00, 3H, s

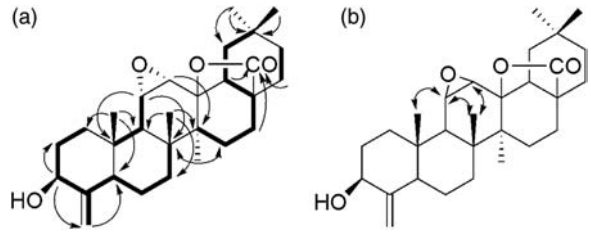

Figure 2. (a) COSY (bold lines) and key HMBC (arrows) correlations of **1**; (b) selected NOE experiments of **1**.

at δ 29.6, C-8 at δ 41.3, C-9 at δ 48.6, and C-14 at δ 40.7; H-27 at δ 1.14 correlated with C-8 at δ 41.3, C-14 at δ 40.7, and C-15 at δ 27.0; H-29 at δ 0.93 correlated with C-20 at δ 31.4 and C-19 at δ 37.8; H-30 at δ 1.00 correlated with C-20 at δ 31.4, C-19 at δ 37.8, and C-29 at δ 23.6, suggesting the presence of five methyl groups at C-10, C-8, C-14, C-20, and C-20, respectively. The orientation of H-3 was deduced as α from the large coupling constant at δ 4.04 (1H, dd, $J = 10.8$ and 5.6 Hz), then 3-OH was β-oriented. Moreover, the NOE correlations between H-11 and Me-25, H-11 and Me-26 suggested the configuration of an 11α,12α-epoxide. Thus, the structure of compound **1** was determined as 11α,12α-epoxy-3β-hydroxy-24-nor-olean-4(23)-en-28,13β-olide. The NMR spectral data of compound **1** were similar to those of ilelatifol A [6], but 2-OH was absence in compound **1** compared to ilelatifol A.

Compound **2** (Figure 1) was obtained as colorless powder. Its molecular formula was assigned to be $C_{25}H_{30}O_{12}$ based on (+)-HR-ESI-TOF-MS at *m/z* 545.1636 $[M + Na]^+$. The ^1H NMR spectrum showed nine aromatic proton signals including an *ortho*-disubstituted benzyl signals at δ 7.69 (1H, d, $J = 7.6$ Hz), 7.06 (1H, t, $J = 7.6$ Hz), 7.50 (1H, t, $J = 7.6$ Hz), 7.24 (1H, d, $J = 7.6$ Hz) and a mono-substituted benzyl signals at δ 7.49 (2H, d, $J = 7.2$ Hz), 7.38 (2H, t, $J = 7.2$ Hz), and 7.33 (1H, t, $J = 7.2$ Hz) by the analysis of ^1H–^1H COSY correlations. The signals at δ 5.36 (2H, br.s), 5.44 (1H, br.s), 5.11 (1H, d, $J = 7.2$ Hz) and 11 overlapped signals at δ 3.44–3.99 inferred the presence of one oxygenated methylene group and two sugar moieties. The ^{13}C NMR spectrum exhibited 25 signals, of which one carbonyl carbon signal at δ 168.2 and 12 aromatic carbon signals were assigned to the aglycone, and the remaining signals belonged to the two sugar moieties. ^1H and ^{13}C NMR spectral data of **2** were assigned based on ^1H NMR, ^{13}C NMR, ^1H–^1H COSY, HSQC, HMBC, and 1D-TOCSY analyses (Table 2). In HMBC spectrum, H-6 at δ 7.69 correlated with C-7 at δ 168.2 and C-2 at δ 157.6; H-3 at δ 7.24 correlated with C-2 at δ 157.6, C-5 at δ 122.7, and C-1 at δ 123.0; H-7′ at δ 5.36 correlated with C-7 at δ 168.2, C-1′ at δ 137.6, and C-2′, 6′ at δ 129.3, indicating that the benzyl group was linked to C-7, and the structure of aglycone is benzyl 2-hydroxy-benzoate. Analysis of the ^1H and ^{13}C NMR spectral data from the sugar moiety indicated the existence of one hexose and one pentose, which was further confirmed by the detection of glucose and apiose after acid hydrolysis of **2**. D-Glucose was determined by measuring the optical rotation as dextrorotation after acid hydrolysis and separation, and the anomeric proton of glucose at δ 5.11 (1H, d, $J = 7.2$ Hz) suggested its β-configuration. The remaining five carbon signals at δ 111.2, 78.0, 80.3, 75.1, and 65.6 could be assigned to another sugar apiose [7] from HMBC and COSY correlations (Figure 3). The orientation of apiose was deduced as β by the singlet of anomeric proton, while α-orientation should be the doublet ($J = 4.5$ Hz) [8]. D-Configuration was determined by comparing the anomeric carbon signal with literature, in

Table 2. ^{1}H and ^{13}C NMR spectral data for **2** (MeOD, TMS, δ).

	2		
Position	δ_C, DEPT	δ_H, Mult. (*J* in Hz)	HMBC correlation
1	123.0, C		
2	157.6, C		
3	116.7, CH	7.24, d (7.6)	C-1/C-2/C-4/C-5
4	134.4, CH	7.50, t (7.6)	C-2/C-3/C-5/C-6
5	122.7, CH	7.06, t (7.6)	C-1/C-3/C-4/C-6
6	131.8, CH	7.69, d (7.6)	C-7/C-2/C-4
7	168.2, C		
1′	137.6, C		
2′	129.3, CH	7.49, d (7.2)	C-7′/C-4′/C-3′,5′
3′	129.5, CH	7.38, t (7.2)	C-1′/C-4′/C-2′,6′
4′	129.1, CH	7.33, t (7.2)	C-2′,6′
5′	129.5, CH	7.38, t (7.2)	C-1′/C-4′/C-2′,6′
6′	129.3, CH	7.49, d (7.2)	C-7′/C-4′/C-3′,5′
7′	68.0, CH_2	5.36, br.s	C-7/C-1′/C-2′,6′
1″	100.8, CH	5.11, d (7.2)	C-2/C-3″
2″	80.1, CH	3.67, dd (7.6,7.2)	C-1″/C-3″/C-4″
3″	77.9, CH	3.61, t (7.6)	C-4″/C-2″/C-5″
4″	71.1, CH	3.44, m	C-2″/C-3″/C-5″
5″	78.0, CH	3.44, m	C-3″/C-6″
6″	62.4, CH_2	3.87, d (9.2)	C-5″
		3.69, d (9.2)	C-5″
1‴	111.2, CH	5.44, br.s	C-2″/C-4‴
2‴	78.0, CH	3.99, br.s	C-1‴/C-4‴/C-5‴
3‴	80.3, C		
4‴	75.1,CH_2	3.90, d (9.0)	C-5‴/C-2‴/C-1‴
		3.69, d (9.0)	C-5‴/C-2‴
5‴	65.6, CH_2	3.53, s	C-2‴/C-3‴/C-4‴

which β-D configuration located at δ 110.2 while β-L at δ 105.4 [9]. In the HMBC spectrum, H-1″ at δ 5.11 correlated with C-2 at δ 157.6, H-1‴ at δ 5.44 correlated with C-2″ carbon signal at δ 80.1, indicating that the apiosyl group was linked to C-2″ of glucose, and the glucosyl group was linked to C-2 of the aglycone. Thus, the structure of **2** was determined as benzyl 2-*O*-β-D-apiofuranosyl-(1 → 2)-β-D-glucopyranosyl benzoate. The NMR spectral data of **2** were similar to those of benzyl 2-*O*-β-D-apiofuranosyl-(1 → 2)-β-D-glucopyranosyl-2,6-dihydroxy-benzoate, especially in B-ring and disaccharide moiety, the difference of NMR data in A-ring was caused by the lack of 6-OH in **2**, compared to the literature [10].

Compounds **3**–**10** were identified as friedelin [11], 3beta-acetoxy-hop-22(29)-ene [12], 3beta-friedelinol [13], 3beta-hydroxyglutin-5-ene [14], 3beta-hydroxy-hop-22(29)-ene [15], oleanolic acid [16], ursolic acid [17], and squalene [18], respectively, on the basis of spectroscopic analysis.

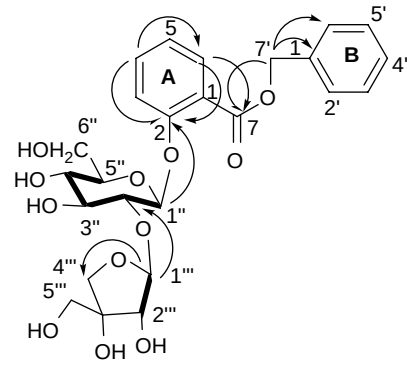

Figure 3. COSY (bold lines) and key HMBC (arrows) correlations of **2**.

Table 3. Anti-HCV activities of selected compounds.

	HCV inhibition (%)		VSV inhibition (%)	
Compound	1 μM	5 μM	1 μM	5 μM
Echincystic acid	10.33	53.58	0	0
1	0	32.45	0	0
3	0	0	15.94	19.43
4	0	5.12	20.41	28.02
5	0.84	4.19	26.47	26.49
6	13.66	37.06	37.30	27.06
7	0	9.16	24.15	22.22

Reference compound.

Pentacyclic triterpenoids had been reported possessing the anti-HCV activity. Thus, anti-HCV activity of compounds **1** and **3**–**7** was evaluated using the method reported [4] and echincystic acid as reference compound. The results were summarized in Table 3. None of the compounds showed anti-HCV activity.

3. Experimental

3.1 General experimental procedures

UV spectra were acquired on a Cary 300 spectrophotometer (Varian, Palo Alto, CA, USA). IR data were measured on a Nicolet NEXUES-470 FT-IR instrument (Nicolet, Austin, TX, USA). HR-ESI-TOF-MS were recorded on a Bruker APEX IV FT-MS (7.0T) mass spectrometer (Bruker, Billerica, MA, USA). 1D and 2D NMR spectra were taken on a Bruker AV 400 spectrometer (Bruker, Fllanden, Switzerland) with tetramethylsilane (TMS) as internal standard. Thin layer chromatography (TLC) and column chromatography (CC) were performed on silica gel plates and silica gel G separately (TLC: GF_{254} and CC: 200–300 mesh; Qingdao Marine Chemical Co., Ltd, Qingdao, China), Sephadex LH-20 (GE Healthcare, Uppsala, Sweden), and SciBioChem MCI GEL (SBC MCI GEL, 75–100 μm, Chengdu Kepubio Co., Ltd., Chengdu, China). Solvents were of analytical grade, which were purchased from Beijing Chemical Corporation (Beijing, China). Bright-Glo reagent was purchased from Promega (Madison, WI, USA). Fractions were monitored by TLC, and spots were visualized on precoated silica gel plates by spraying 10% H_2SO_4 in ethanol followed by heating.

3.2 Plant material

The whole plants were collected from Liuzhou city, Guangxi Province of China in July 2009 and identified as *Pilea cavaleriei Levi.* subsp. *cavaleriei* by Prof. De-Hai Qin, Guangxi Institute of Traditional Chinese Medicine. A specimen (no. NME20090701) is deposited at the Department of Natural Medicines, School of Pharmaceutical Sciences, Peking University Health Science Center.

3.3 Extraction and isolation

The air-dried and powdered whole plants of *P. cavaleriei* (10 kg) were percolated with 95% aqueous EtOH and 50% aqueous EtOH at room temperature, respectively. After evaporation of the solvent under reduced pressure, the residues were mixed and suspended in water and then successively partitioned with petroleum ether, EtOAc, and *n*-BuOH to afford 235.0 g, 35.0 g, and 175.0 g of extracts, respectively. The petroleum ether extract (232.0 g) was subjected to silica gel CC and eluted with petroleum ether–acetone (20:1 to 1:5, v/v) to give eight fractions (Fr.1–Fr.8). Fr. 5 (27.0 g) was further separated on MCI CC eluted with MeOH–H_2O (70:30 to 100:0, v/v) to yield four subfractions (Fractions A–D). Fraction

B (80% aqueous MeOH extracts, 2.3 g) was separated on silica gel CC eluted with petroleum ether–acetone (4:1, v/v), and then purified by Sephadex LH-20 CC eluted with $CHCl_3$–MeOH (1:1) to afford **1** (30.0 mg). Fr. 8 (32.0 g) was further separated on MCI CC eluted with MeOH–H_2O (50:50 to 100:0, v/v) to get five subfractions (Fractions I–V). Fraction III (70% aqueous MeOH extracts, 4.2 g) was separated on silica gel CC eluted with petroleum ether–acetone (10:1, v/v), and then purified by Sephadex LH-20 CC eluted with MeOH to afford **2** (12.0 mg). Fr. 1 (21.0 g) was further separated on repeated silica gel CC eluted with petroleum ether–acetone (50:1 to 5:1, v/v) to afford **3** (500.0 mg), **4** (8.0 mg), **5** (20.0 mg), and **10** (20.0 mg). Fr. 2 (14.2 g) was separated on repeated silica gel CC eluted with petroleum ether–acetone (30:1 to 3:1, v/v), and then purified by Sephadex LH-20 CC eluted with $CHCl_3$–MeOH (1:1) to afford **6** (15.0 mg). Fr. 3 (36.6 g) was separated on repeated silica gel CC eluted with petroleum ether–acetone (10:1–1:3, v/v), and then purified by Sephadex LH-20 CC eluted with ($CHCl_3$–MeOH, 1:1) to afford **7** (12.0 mg). Fr. 6 (21.0 g) was further separated on MCI CC eluted with MeOH–H_2O (70:30 to 100:0, v/v), and then purified by Sephadex LH-20 CC eluted with MeOH to afford **8** (10.0 mg) and **9** (20.0 mg).

*3.3.1 11α,12α-Epoxy-3β-hydroxy-24-nor-olean-4(23)-en-28,13β-olide (**1**)*

Colorless powder; IR (KBr) ν_{max} (cm^{-1}): 3495, 2942, 1758, 1649, 1146, and 931; for ^1H and ^{13}C NMR spectral data, see Table 1; EI-MS *m/z* 454 $[M]^+$; HR-ESI-MS *m/z* 455.3161 $[M + H]^+$ (calcd for $C_{29}H_{43}O_4$, 455.3161).

*3.3.2 Benzyl 2-O-β-D-apiofuranosyl-(1 → 2)-β-D-glucopyranosyl benzoate (**2**)*

Colorless powder; UV λ_{MeOH} (nm) (log ϵ): 286 (3.98); IR (KBr) ν_{max} (cm^{-1}): 3392, 2930, 1715, 1600, 1490, 1251, and 1076; for ^1H and ^{13}C NMR spectral data, see Table 2; ESI-MS *m/z* 545 $[M + Na]^+$; HR-ESI-MS *m/z* 545.1636 $[M + Na]^+$ (calcd for $C_{25}H_{30}O_{12}Na$, 545.1635).

3.4 Acid hydrolysis and analysis of sugars of compound 2

Compound **2** (4.0 mg) was hydrolyzed with 5% HCl (3 ml) at 70°C for 6 h. After being cooled, the pH value of reaction mixture was adjusted to 7.0, and then subjected the mixture to Sephadex LH-20 CC eluted with MeOH to afford glucose and apiose (not pure). The sugars were identified as glucose by co-TLC (BuOH:CH_3COOH:H_2O, 4:2:1) with authentic glucose and reference apiose, obtained from the hydrolysis of authentic (−)-syringaresnol-4-*O*-β-D-apiofuranosyl-(1 → 2)-β-D-glucopyranoside.

3.5 Anti-HCVpp infection assays

For compound library screening, infections were performed in 96-well plates by mixing HCVpp or VSVpp with 5×10^3 Huh-7 cells/well in the presence or absence of test inhibitors, followed by incubation at 37°C. Luciferase activity, reflecting the degree of the pseudoparticles into host cells, was measured after the infection of 3 days using the Bright-Glo reagent (Promega). Test compounds were serially diluted to give a final concentration at 1 and 5 μM in 1% dimethyl sulfoxide (DMSO). Maximum activity (100% of control) and background were derived from control wells containing DMSO alone or from uninfected wells, respectively. The individual signals in each of the compound test wells were then divided by the averaged control values (wells lacking inhibitor), after background subtraction, and multiplied by 100% to determine percent activity. The corresponding % inhibition values were then calculated by subtracting this value from 100. The specificity of the compounds for inhibiting HCV was determined by evaluating the inhibition of VSVpp infection in parallel.

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