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Sesquiterpenoids from *Pilea cavaleriei* subsp. *crenata*

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ABSTRACT

Three new humulane-type sesquiterpenes, 8-*O*-(*p*-coumaroyl)-5β-hydroperoxy-1(10)*E*,4(15)-humuladien-8α-ol (**1**), 8-*O*-(3-nitro-*p*-coumaroyl)-1(10)*E*,4(15)-humuladien-5β,8α-diol (**2**) and 8-*O*-(*p*-coumaroyl)-1(10)*E*,4(5)*E*-humuladien-8-ol (**3**), and a new copaborneol derivative, 1-*O*-*p*-coumaroyl-copaborneol (**4**), have been isolated from the methanol extract of *Pilea cavaleriei* Lévl. subsp. *crenata* C. J. Chen. Their structures were elucidated using spectroscopic methods. Cytotoxic and antimicrobial activities of the isolated compounds were evaluated.

Pilea is a large genus (about 400 species) belonging to the Urticaceae family and widely distributed in tropical and subtropical regions of the world.[1] In China, approximately thirty *Pilea* species and subspecies are used as traditional Chinese medicines.[2] *Pilea cavaleriei* Lévl. subsp. *crenata* C. J. Chen, a common Zhuang folk medicine, are used to treat burn wounds, swelling and cough, clear sputum, relieve pain, and so on.[3] However, its chemical constituents are not clear yet. In this letter, the isolation and structure elucidation of three new humuladiene derivatives (**1**–**3**) and a new copaborneol derivative (**4**) (Fig. 1) from the whole plant are reported, together with the results of biological assays.

Compound **1**[4] was obtained as colorless oil. Positive HRESIMS analysis of the compound exhibited a pseudomolecular ion peak at *m*/*z* 423.2163 $[M+Na]^+$ (calcd, 423.2147), allowing the molecular formula $C_{24}H_{32}O_5$ (Ω, 9) to be determined. The IR spectrum of **1** indicated the possible presence of hydroxy groups (3399 cm^{-1}), a carbonyl group (1704 cm^{-1}), double bonds (1680 and 1631 cm^{-1}), and a phenyl ring (1605, 1587 and 1515 cm^{-1}). The ^1H and ^{13}C NMR spectra of **1** (Table 1) displayed signals for a 4-hydroxyphenyl group [δ_H 7.41 (2H, d, J = 8.5 Hz, H-2′ and H-6′) and 6.85 (2H, d, J = 8.5 Hz, H-3′ and H-5′), δ_C 158.0 (C-4′), 130.0 (C-2′ and C-6′), 115.9 (C-3′ and C-5′) and 127.0 (C-1′)], a *trans* olefin bond [δ_H 7.60 (d, J = 15.9 Hz, H-7′) and 6.26 (d, J = 15.9 Hz, H-8′); δ_C 144.5 (C-7′) and 115.7 (C-8′)], and an ester carbonyl group [δ_C 167.2 (C-9′)], which were assigned a *p*-coumaroyl group. In addition, its ^1H NMR spectrum showed the presence of an exocyclic double bond [δ_H 5.19 (s) and 5.16 (s)], a trisubstituted double bond [δ_H 5.31 (t, J = 5.1 Hz, H-1)], and three methyl groups [δ_H 1.74 (3H, s, H-14), 0.99 (3H, s, H-13), and 0.83 (3H, s, H-12)]. Signals for two oxygenated methines [δ_C 85.2 (C-5), 70.2 (C-8)], a quaternary carbon [δ_C 32.3 (C-11)], and five methylenes [δ_C 46.2 (C-9), 43.7 (C-7), 40.2 (C-6), 37.5 (C-3), 30.0 (C-2)] were observed in the ^{13}C NMR spectra of **1**. According to the above analyses, except for the *p*-coumaroyl moiety (Ω, 6), the remaining moiety including 15 carbon atoms was suggested as a monocyclic sesquiterpenoid skeleton by calculating the degree of unsaturation. As well known, the carbon signal of the oxygenated methine at δ_C 85.2 is rather low for ordinary hydroxymethine, the group was therefore determined as a hydroperoxymethine.[5] The obtained molecular formula ($C_{24}H_{32}O_5$) also confirmed the presence of a hydroperoxy group (OOH).

The ^1H–^1H COSY spectrum exhibited three partial structures (Fig. 2), **a** (C-1 to C-3), **b** (C-5 to C-6), and **c** (C-7 to C-9). Based on the HMBC spectrum (Fig. 2) correlations of H_2-15 to C-3 and C-5, H_3-12 and H_3-13 to C-6 and C-7, and H_3-14 to C-1 and C-9, fragments **a**, **b** and **c** were connected to form a carbon skeleton of humulane-type sesquiterpenoids with an eleven-membered

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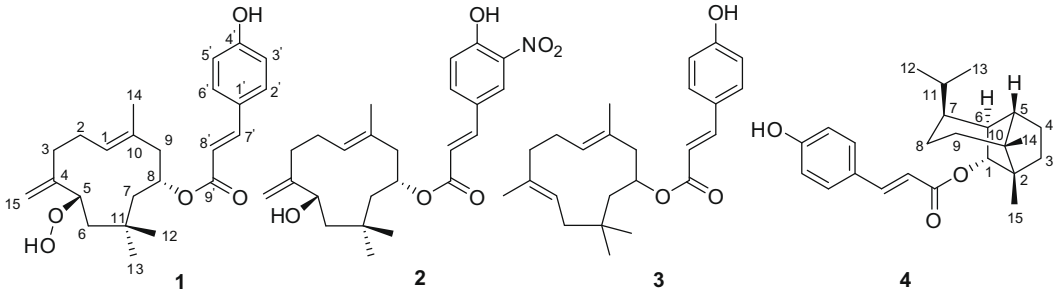

Figure 1. Chemical structures of compounds **1**–**4**.

macrocycle. The linkage of the *p*-coumaroyl substituent to C-8 was established by the correlation of H-8 to C-9′. An *E*-configuration for the 1,10-double bond was determined by the ROESY correlation of H-1/H-9. ROESY correlation of H-5/CH_3-13 showed that these protons were cofacial, and were arbitrarily assigned as the α-orientation, while 5-OOH and CH_3-12 were β-oriented. The correlation of H-8/CH_3-12 deduced H-8 to be the β-orientation. Therefore, **1** was elucidated as 8-*O*-(*p*-coumaroyl)-5β-hydroperoxy-1(10)*E*,4(15)-humuladien-8α-ol.

Compound **2**[6] was gained as yellow oil. Its molecular formula was established as $C_{24}H_{31}NO_6$ on the basis of the HRESIMS. The IR spectrum of **2** indicated the possible presence of hydroxy groups (3439 cm^{-1}), a carbonyl group (1707 cm^{-1}), double bonds and a phenyl ring (1625, 1580 and 1491 cm^{-1}), and a nitro group (1539 and 1316 cm^{-1}). The UV spectrum showed absorption maxima at 379 (3.36) and 284 (4.37) nm resembling those of *o*-nitrophenols.[7] A 3-nitro-*p*-coumaroyl moiety was further confirmed by the presence of an aromatic ABX coupling system [δ_H 8.27 (d, *J* = 2.1 Hz, H-2), 7.90 (dd, *J* = 8.8 and 2.1 Hz, H-6), and 7.17 (d, *J* = 8.8 Hz, H-5)], the signals for a *trans* double bond [δ_H 7.64 (d, *J* = 16.0 Hz, H-7′) and 6.48 (d, *J* = 16.0 Hz, H-8′)], and a ester carbonyl signal at δ_C 169.7 (C-9) in the NMR spectra of **2** (Table 1). The remaining 15 NMR carbon signals are very similar to those in the sesquiterpenoid moiety of **1**, except that the hydroperoxymethine signal at δ_C 85.2 (CH, C-5) in **1** is replaced by a hydroxymethine signal at δ_C 72.2 (CH, C-5) in **2**. Based on 2D NMR correlations, the sesquiterpenoid moiety of **2** was elucidated as 1(10)*E*,4(15)-humuladien-5β,8α-diol, and the 3-nitro-*p*-coumaroyl group was located at C-8. Accordingly, compound **2** was identified as 8-*O*-(3-nitro-*p*-coumaroyl)-1(10)*E*,4(15)-humuladien-5β,8α-diol.

The molecular formula of compound **3**[8] was confirmed as $C_{24}H_{32}O_3$ by the HRESIMS. The ^1H and ^{13}C NMR spectra of **3** (Table 1) are closely similar to those of **1** except that the signals for the methylene [δ_H 5.19 (s, H-15) and 5.16 (s, H-15); δ_C 115.0 (CH_2,

Table 1
NMR Spectroscopic data for compounds **1**–**4**

Position	**1**[a]		**2**[b]		**3**[a]		**4**[c]	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	127.4	5.31, t (5.1)	129.3	5.39, t (7.3)	127.6	4.97, m	87.8	4.59, s
2	30.0	2.30, m	30.4	2.34, m	25.1	2.32, m	48.8	
		2.21, m		2.16, m		2.12, m		
3	37.5	2.52, m	36.8	2.37, m	39.3	2.18, m	27.4	1.92, m
		2.51, m		2.23, m		2.06, m		1.31, m
4	152.3		157.0		134.6		24.9	1.76, m
								1.24, m
5	85.2	4.26, d (7.6)	72.2	3.92, d (8.1)	124.4	4.89, m	41.4	1.68, d (5.1)
6	40.2	1.45, 2H, m	45.1	1.64, d (14.7) 1.32, m	38.8	2.06, m 1.80, d (14.3)	49.9	1.44, m
7	43.7	1.90, d (15.5), 1.60, dd (15.5, 5.1)	44.5	1.96, d (15.8) 1.57, m	42.7	1.88, d (15.3), 1.47, dd (15.3, 8.0)	47.5	1.55, d (7.3)
8	70.2	4.79, m	72.1	4.82, m	72.7	4.63, m	23.4	1.52, m
								1.22, m
9	46.2	2.29, m	46.8	2.23, m	46.2	2.18, m	29.0	1.44, m
		2.07, m		2.07, m		2.04, m		1.28, m
10	131.5		132.1		131.7		49.5	
11	32.3		33.5		33.6		31.8	1.44, m
12	27.6	0.83, 3H, s	28.6	0.82, 3H, s	27.1	0.88, 3H, s	20.4	0.86, 3H, d (6.9)
13	28.5	0.99, 3H, s	29.4	1.01, 3H, s	30.8	0.99, 3H, s	20.6	0.86, 3H, d (6.9)
14	17.1	1.74, 3H, s	17.6	1.73, 3H, s	17.9	1.73, 3H, s	19.3	0.87, 3H, s
15	115.0	5.19, s	112.3	5.06, s	15.9	1.50, 3H, s	13.7	0.89, 3H, s
		5.16, s		4.96, s				
1′	127.0		128.0		127.0		127.2	
2′	130.0	7.41, d (8.5)	126.6	8.27, d (2.1)	130.0	7.42, d (8.6)	129.9	7.44, d (8.6)
3′	115.9	6.85, d (8.5)	136.3		115.9	6.86, d (8.6)	115.8	6.85, d (8.6)
4′	158.0		157.0		158.0		157.7	
5′	115.9	6.85, d, (8.5)	121.8	7.17, d (8.8)	115.9	6.86, d (8.6)	115.8	6.85, d (8.6)
6′	130.0	7.41, d (8.5)	136.1	7.90, dd (8.8, 2.1)	130.0	7.42, d (8.6)	129.9	7.44, d (8.6)
7′	144.5	7.60, d (15.9)	143.4	7.64, d (16.0)	144.4	7.61, d (15.9)	143.9	7.60, d (16.0)
8′	115.7	6.26, d (15.9)	119.6	6.48, d (16.0)	116.0	6.27, d (15.9)	116.2	6.33, d (16.0)
9′	167.2		169.7		167.5		168.0	

[a] Measured in $CDCl_3$ at 400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR.
[b] Measured in CD_3OD at 500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR.
[c] Measured in $CDCl_3$ at 500 MHz for ^1H NMR and 100 MHz for ^{13}C NMR.

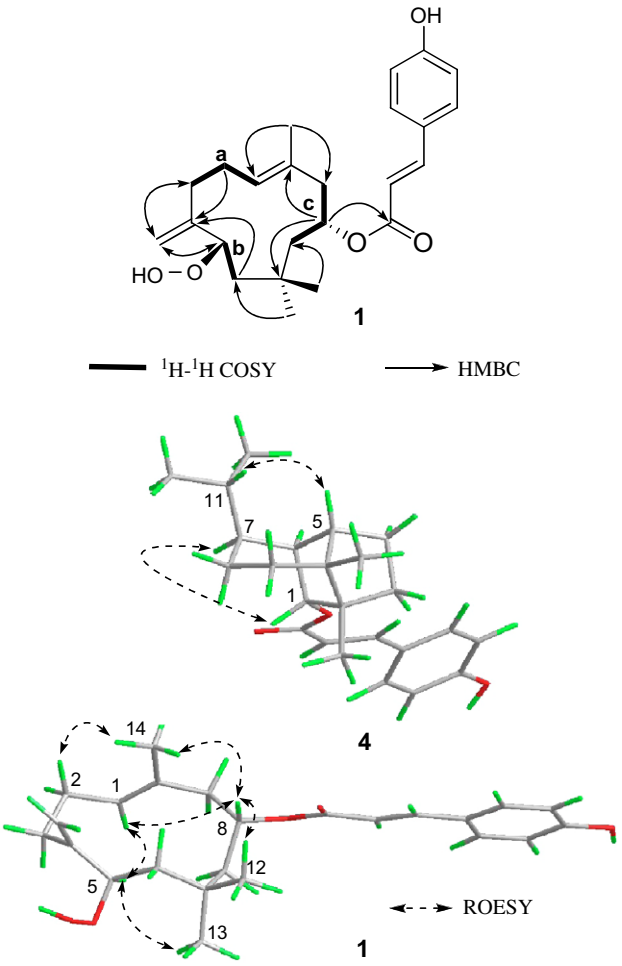

Figure 2. Key 2D NMR correlations of **1** and **4**.

C-15)] and hydroperoxymethine [δ_H 4.26 (d, J = 7.6, H-5); δ_C 85.2 (CH, C-5)] groups in **1** are replaced by those for a methyl group [δ_H 1.50 (3 H, s, H-15); δ_C 15.9 (CH_3, C-15)] and an olefinic methine carbon [δ_H 4.89 (1H, H-5, s), δ_C 124.4 (C-5)] in **3**, respectively. This signifies that the hydroperoxy group is eliminated and the exocyclic double bond is migrated into the ring in compound **3**. Both 1,10- and 4,5-double bonds of **3** were assigned as *E*-configuration by ROESY spectrum of **3**. Therefore, compound **3** was assigned as 8-*O*-(*p*-coumaroyl)-1(10)*E*,4(5)*E*-humuladien-8-ol. This compound might be a racemoid because its optical rotation value was zero.

Compound **4**[9] was determined to have the molecular formula $C_{24}H_{32}O_3$ (Ω, 9), on the basis of the HRESIMS. According to the NMR spectra (Table 1) and the unsaturation degree of compound **4**, it was elucidated to possess a *p*-coumaroyl group and a tricyclic sesquiterpenoid moiety. The later was elucidated as copaborneol by comparison of its NMR spectra data with those reported in the literature.[10] The *p*-coumaroyl substituent was located at C-1 by the HMBC correlation of H-1 to C-9′. The relative configuration of copaborneol, which was not integrated in the literature, was established by the ROESY correlations of **4** as showed in Figure 2. Consequently, compound **4** was deduced as 1-*O*-*p*-coumaroyl-copaborneol.

The isolated compounds were evaluated in vitro for their cytotoxicity against proliferation of seven human tumor cell lines (K562, SGC-7901, AGZY, BIU-87, EJ, SK-OV-3, and GLC-82) using the improved MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method.[11] In addition, compounds **1** and **3** were also tested for their cytotoxic properties on A549 and MCF-7 cell lines with the sulforhodamine B (SRB) assay[12] using 5-fluorouracil and adriamycin as the positive control, respectively. Further, the activity of compounds **1** and **3** against *Escherichia coli* and *Staphylococcus aureus* were measured by the microdilution assays with gentamicin as positive control.[13] The results revealed that compound **3** exhibited weak activities against K562 (IC_{50} = 12.01 μg/mL), AGZY (IC_{50} = 27.82 μg/mL), and A549 (IC_{50} = 25.60 μg/mL) cell lines (Table 2).

Table 2
Cytotoxic (IC_{50} in μg/mL) and antibacterial (MIC in μg/mL) activities of **1**–**4**[a]

Cells and bacteria	1	2	3	4	Positive control
K562	57.39	na	12.01	41.72	0.32[b]
SGC-7901	na	na	49.42	na	3.00[b]
AGZY	na	na	27.82	na	3.38[b]
BIU-87	na	na	na	na	4.52[b]
EJ	na	na	na	na	4.67[b]
SK-OV-3	na	na	na	na	3.38[b]
GLC-82	na	na	na	na	2.88[b]
A549	33.35	nt	25.60	nt	2.00[c]
MCF-7	23.39	nt	40.51	nt	5.00[d]
E. coli	na	nt	na	nt	0.05[e]
S. aureus	45.0	nt	na	nt	0.05[e]

[a] K562 (human chronic myelogenous leukemia cell line); SGC-7901 (human stomach cancer cell line); AGZY (low-metastatic human lung adenocarcinoma cell line); BIU-87 (human bladder transitional carcinoma cell line); EJ (human bladder cancer cell line); SK-OV-3 (human ovarian carcinoma cell line); GLC-82 (human glandular lung cancer cell line); A549 (human lung adenocarcinoma cell line); MCF-7 (human breast cancer cell line); na = no activity; nt = not tested.
[b] *cis*-Platin as positive control.
[c] 5-Fluorouracil as positive control.
[d] Adriamycin as positive control.
[e] Gentamicin as positive control.

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Supplementary data

Supplementary data (general experimental procedures, plant material, extraction and isolation of compounds, 1D and 2D NMR spectra of compounds **1**–**4**, and brief introduction of the MTT method) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.08.001.

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4. *Compound* **1**: colorless oil ($CHCl_3$); $[\alpha]_D^{22}$ +12.9 (*c* 0.27, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 310 (3.98), 301 (3.97), 228 (3.48), 217 (3.54), 211 (3.60), 206 (3.62), 200 (3.63), 194 (3.64) nm; IR (KBr) ν_{max} 3399, 1704, 1680, 1631, 1605, 1587, 1515, 1444, 1168 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); ESIMS *m*/*z* 423 $[M+Na]^+$; HRESIMS *m*/*z* 423.2163 $[M+Na]^+$ (calcd for $C_{24}H_{32}O_5Na$, 423.2147).

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6. *Compound* **2**: yellow oil ($CHCl_3$); $[\alpha]_D^{21}$ +65.5 (c 0.15, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 379 (3.36), 284 (4.37), 233 (4.10), 221 (4.12), 201 (4.18), 194 (4.18) nm; IR (KBr) ν_{max} 3439, 1707, 1625, 1580, 1539, 1491, 1316, 1180 cm^{-1}; ^1H and ^{13}C NMR, (see Table 1); ESIMS *m*/*z* 452 $[M+Na]^+$; HRESIMS *m*/*z* 452.2041 $[M+Na]^+$ (calcd for $C_{24}H_{31}NO_6Na$, 452.2049).
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8. *Compound* **3**: colorless oil ($CHCl_3$); $[\alpha]_D^{22}$ 0 (*c* 0.54, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 309 (4.04), 300 (4.03), 221 (3.62), 207 (3.73), 204 (3.74) nm; IR (KBr) ν_{max} 3387, 1705, 1675, 1631, 1605,1586, 1515, 1173 cm^{-1}; ^1H and ^{13}C NMR, (see Table 1); EIMS *m*/*z* 368 $[M]^+$ (3), 204 (26), 189 (19), 175 (13), 161 (27), 147 (100), 119 (24), 91 (25); HRESIMS *m*/*z* 391.2255 $[M+Na]^+$ (calcd for $C_{24}H_{32}O_3Na$, 391.2249).
9. *Compound* **4**: colorless oil ($CHCl_3$); $[\alpha]_D^{22}$ +9.3 (c 0.36, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 308 (3.92), 299 (3.92), 223 (3.49), 220 (3.52), 203 (3.63), 197 (3.64) nm; IR (KBr) ν_{max} 3424, 1677, 1632, 1606, 1515, 1168 cm^{-1}; ^1H and ^{13}C NMR, (see Table 1); EIMS *m*/*z* 368 $[M]^+$ (2), 221 (6), 204 (16), 161 (10), 147 (100), 119 (10), 91 (10); HRESIMS *m*/*z* 391.2253 $[M+Na]^+$ (calcd for $C_{24}H_{32}O_3Na$, 391.2249).
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