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Humulane-type sesquiterpenoids from *Pilea cavaleriei* subsp. *crenata*†

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Cang-Song Liao, Chun-Ping Tang, Sheng Yao and Yang Ye*

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Nine new, uncommon humulane-type sesquiterpenoids (**1**, **2**, **4**, **6–11**), together with two known derivatives, were isolated from extracts of the plant *Pilea cavaleriei* subsp. *crenata*. The structures of these compounds were fully elucidated by extensive analyses of spectroscopic data (MS, 1D- and 2D-NMR), use of the Mosher method, and by X-ray crystallographic analysis, in combination with chemical conversions. An ene reaction was discovered during the chemical transformations, which might provide an explanation for the wide distribution of the allylic hydroperoxide group in natural products.

Introduction

Sesquiterpenoids, derived from farnesyl pyrophosphate (FPP), refer to a class of C-15 compounds comprised of three isoprene units. Found mainly in higher plants, sesquiterpenoids have an extensive range of complex structures[1–3] with a variable core ring system and various bioactivities, especially anticancer activity.[4]

Found in plants,[5–8] liverworts,[9] and fungi,[10] humulane-type sesquiterpenoids represent an uncommon type of compound possessing a characteristic 11-membered ring in the molecule, which is very challenging for structural elucidation, particularly for determination of the absolute configuration, due to the flexible macrocycle. In 2009, Tang and co-workers[11] identified three humulane-type sesquiterpenoids from *Pilea cavaleriei* subsp. *crenata*, belonging to the Urticaceae family, but the absolute configuration of these compounds has not been fully determined. Since plants of the genus *Pilea* are reported to exhibit antidiabetic[12] and antimicrobial[13] activities, a systematic investigation on the chemical constituents of *Pilea cavaleriei* subsp. *crenata* was carried out.

Herein, we report the isolation of nine new compounds (**1**, **2**, **4**, **6–11**), along with two known derivatives (**3**, **5**), and their structural elucidation using 1D- and 2D-NMR spectroscopy, the Mosher method, and single-crystal X-ray diffraction analysis, as well as chemical conversions (Fig. 1).

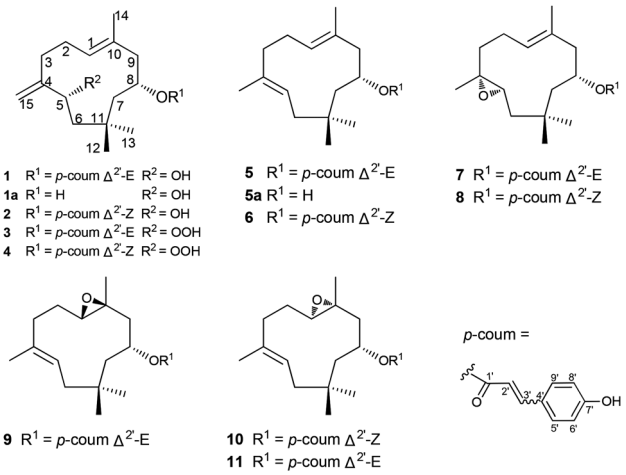


Fig. 1 Structure of isolated and synthetic compounds.

Results and discussion

The aerial part of *P. cavaleriei* was extracted with ethanol. The ethanol extract was suspended in water and partitioned with petroleum ether to give a PE extract. The PE extract was again partitioned with methanol to give a MeOH extract, which was then isolated by repeated column chromatography over silica gel, Sephadex LH-20 and MCI, and preparative HPLC to afford pure compounds.

The molecular formula of **1** was determined to be $C_{24}H_{32}O_4$ from the quasi-molecular ion peak at *m*/*z* 383.2238 $[M - H]^-$ (calcd 383.2222) in the ESI-HRMS, corresponding to nine degrees of unsaturation. The ^{13}C NMR (Table 1) and DEPT spectra showed 24 resonances ascribed to six quaternary, nine methine, six methylene, and three methyl carbons. The ^1H NMR spectrum (Table 2) displayed signals of a

State Key Laboratory of Drug Research, and Department of Natural Products Chemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu-Chong-Zhi Road, Zhangjiang Hi-Tech Park, Shanghai 201203, P. R. China. E-mail: yye@mail.shcnc.ac.cn; Fax: +86-21-50807088; Tel: +86-21-50806726

†Electronic supplementary information (ESI) available: Spectroscopic data, including ^1H NMR, ^{13}C NMR, ^1H–^1H COSY, HSQC, HMBC, ROESY, and ESI-HRMS, for compounds **1**, **1a**, **2**, **4**, **6–11**. CCDC 925951. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3ob40872h

Table 1 ^{13}C NMR data for compounds **1**, **1a**, **2**, **4**, **6–11** in $CDCl_3$ (δ in ppm)

Position	1	1a	2	4	6	7	8	9	10	11
1	127.7	127.1	127.3	127.5	127.9	126.8	126.9	62.4	63.2	63.2
2	29.7	29.6	29.6	30.1	25.3	24.8	24.8	24.9	24.0	24.2
3	36.3	36.2	36.2	37.6	39.5	38.0	38.0	37.0	37.2	37.5
4	156.3	156.4	156.1	152.4	134.3	60.8	60.7	134.2	134.0	134.2
5	71.9	71.8	72.0	85.4	124.5	62.1	62.0	123.7	123.3	123.5
6	45.0	48.0	44.9	40.4	39.5	36.2	36.2	43.3	37.5	37.7
7	43.8	45.1	43.5	43.6	42.6	43.6	43.4	43.2	45.6	46.1
8	70.4	67.9	70.3	70.1	72.7	70.1	69.7	72.0	68.2	68.8
9	46.3	49.8	46.1	46.2	46.2	46.7	46.6	45.3	48.3	48.7
10	131.6	131.8	131.5	131.7	131.7	131.3	131.2	59.2	59.9	60.0
11	32.7	32.6	32.7	32.4	33.7	32.4	32.3	33.6	32.8	33.1
12	28.1	28.6	28.0	27.8	27.0	27.6	27.5	24.1	28.2	28.6
13	29.0	29.3	29.6	28.7	31.0	29.7	28.6	33.7	29.7	30.0
14	17.4	17.9	17.6	17.4	18.2	17.5	17.7	20.5	18.1	18.0
15	112.1	111.9	112.2	115.0	16.1	16.9	16.9	15.9	15.6	15.8
1′	166.9		166.3	166.1	166.7	167.2	166.1	166.8	165.9	167.1
2′	116.3		117.6	117.8	117.8	115.0	117.2	115.7	117.4	116.1
3′	144.2		143.6	143.5	143.8	144.6	143.9	144.4	143.5	144.5
4′	127.5		127.8	127.5	127.9	126.7	127.0	127.0	127.3	127.4
5′/9′	131.6		131.5	132.4	132.2	131.3	132.3	132.4	132.4	130.1
6′/8′	116.0		115.2	115.1	115.2	115.5	115.0	115.8	114.9	116.0
7′	157.7		157.1	156.9	157.1	158.2	157.1	157.8	156.7	157.7

1,4-disubstituted phenyl group [δ_H 7.42 (2 H, d, J = 8.4 Hz), 6.83 (2 H, d, J = 8.4 Hz)] and a double bond conjugated with a carboxy group [δ_H 7.62 (d, J = 16.2 Hz), 6.26 (d, J = 16.2 Hz)] which, in combination with the corresponding ^{13}C resonances inferred from the HSQC spectrum, indicated the presence of an *E*-*p*-coumaroyl group.[14] The remaining ^{1}H and ^{13}C NMR data showed characteristic signals of an exocyclic double bond (δ_C 112.1 and 156.3), a trisubstituted double bond (δ_C 127.7 and 131.6), and two oxygenated C–H moieties (δ_C 70.4 and 71.9). Since the identified functionalities and segments contributed eight degrees of unsaturation, the one remaining was ascribed to the existence of one additional ring.

The structure of **1** was further established by extensive HMBC analysis (Fig. 2). HMBC correlations from H-12 to C-6, 7, 11, and 13, from H-1 to C-2, 3, 10, and 14, from H-8 to C-7, 9, 10, and 11, and from H-15 to C-3, 4, and 5 constructed an 11-membered ring, and the HMBC correlation from H-8 to C-1′ allowed attachment of the *p*-coumaroyl group to C-8. Thus, **1** was identified as a humulane-type sesquiterpenoid. The *E* orientation of the 1,10-double bond was assigned from the crosspeak of H-1/H-9 in the ROESY spectrum. Thus, the planar structure of **1** was proved.

Due to the flexible 11-membered ring in the molecule, the relative configuration of **1** was difficult to concretely establish based on ROESY experiments. Therefore, compound **1** was treated with $LiAlH_4$ to afford the 8-hydroxy derivative **1a**, which crystallized from methanol. Single-crystal X-ray diffraction analysis of **1a** using Cu Kα radiation was then carried out and the absolute configurations of C-5 and C-8 were both established as *R* (Fig. 3). Thus, the structure of compound **1** was fully verified as (1*E*,5*R*,8*R*)-8-*O*-[(*E*)-*p*-coumaroyl)]-5-hydroxyhumula-1(10),4(15)-dien-8-ol.

Compound **2**, an amorphous powder, gave the same molecular formula of $C_{24}H_{32}O_4$ as **1**, according to the ESI-HRMS data. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) of **2** showed high similarities to those of **1**, revealing that these two compounds shared a common sesquiterpenoid skeleton. The major differences were observed for H-2′ and H-3′ [δ_H 6.82 (d, J = 12.7 Hz), 5.74 (d, J = 12.7 Hz)], indicating a *Z*-2′-*p*-coumaroyl group instead of the *E*-isomer.[14] This was also reflected in the chemical shifts of the aromatic protons [δ_H 7.64 (2 H, d, J = 8.6 Hz), 6.81 (2 H, d, J = 8.6 Hz)]. Thus, **2** was identified as the C-2′ configurational isomer of **1**. To further prove the proposed structure, **2** was treated with $LiAlH_4$, which yielded the identical compound **1a** to that obtained from **1**. Accordingly, **2** was identified as (1*E*,5*R*,8*R*)-8-*O*-[(*Z*)-*p*-coumaroyl]-5-hydroxyhumula-1(10),4(15)-dien-8-ol.

Compounds **3** and **4** possessed the same molecular formula of $C_{24}H_{32}O_5$, with nine degrees of unsaturation. Their NMR data (Tables 1 and 2) suggested that **3** and **4** shared a common humulane-type skeleton, but were isomeric at the C-8 substituent, similar to the situation with **1** and **2**. The NMR data of **3** were almost superimposable with those of **1** except for the chemical shift of H-5 (δ 3.94 in **1**, δ 4.25 in **3**), suggesting a hydroperoxy rather than a hydroxy group located at C-5. This proposed structure was consistent with the molecular formula of **3**, with one more oxygen atom than that of **1**. In the event, **3** was identified as a compound previously reported[11] as 8-*O*-(*p*-coumaroyl)-5β-hydroperoxy-1(10)*E*,4(15)-humuladien-8α-ol with the absolute configuration pending, and therefore **4** was designated as its C-2′ configurational isomer.

Taking the biogenesis into consideration, **3** was thought to have the same *R* configuration of C-5 and C-8 as compounds **1** and **2**. To verify this assumption, **3** was treated with $NaBH_4$ to yield a reduced product, which was identical to compound **1** on the basis of the spectroscopic data. Thus, the structure of **3** was fully established, and renamed (1*E*,5*R*,8*R*)-8-*O*-[(*E*)-*p*-coumaroyl]-5-hydroperoxyhumula-1(10),4(15)-dien-8-ol.

Table 2 ^{1}H NMR data for compounds **1**, **1a**, **2**, **4**, **6–11** in $CDCl_3$ (δ in ppm, J in Hz)

Position	1	1a	2	4	6	7	8	9	10	11
1	5.36, br	5.27, dd (7.4, 5.9)	5.32, br	5.31, br s	4.97, m	5.13, m	5.13, m	3.12, d (10.9)	2.70, d (10.9)	2.74, d (10.9)
2	2.32, m 2.20, m	2.32, m 2.22, m	2.32, m 2.20, m	2.30, m 2.21, m	2.32, m 2.12, m	2.35, m 2.12, m	2.35, m 2.12, m	2.08, m 1.47, m	1.97, m 1.49, m	1.99, m 1.53, m
3	2.44, m 2.27, m	2.38, m 2.27, m	2.44, m 2.27, m	2.52, m 2.51, m	2.18, m 2.06, m	2.15, m 1.17, m	2.15, m 1.17, m	2.34, m 2.26, m	2.25, m	2.25, m
5	3.94, m	3.91, d (8.1)	3.94, br s	4.26, d (6.1)	4.89, m	2.60, d (5.0)	2.59, d (3.8)	5.46, m	5.11, d (10.2)	5.16, d (10.2)
6	1.67, d (14.5) 1.32, m	1.55 1.32	1.70, d (14.5) 1.32, m	1.45, m	2.06, m 1.80, m	1.63, m 1.21, m	1.63, m 1.21, m	2.15, m 2.08, m	2.24, m 1.73, d (14.6)	2.29, m 1.78, d (14.6)
7	1.91, d (15.9) 1.57, m	1.92 1.28	1.91, dd (12.6, 12.7) 1.57, m	1.90, m 1.60, m	1.88, m 1.39, dd (15.5, 7.9)	1.87, m 1.66, m	1.87, m 1.66, m	1.84, m 1.64, m	1.60, m 1.48, m	1.63, m 1.48, m
8	4.81, br	3.59, dddd (2.4, 4.6, 6.9, 9.3)	4.81, br	4.79, m	4.61, dd (8.6, 8.8)	4.68, br	4.68, br	5.27, m	4.68, dd (9.1, 7.7)	4.77, dd (9.1, 7.7)
9	2.23, m 2.07, dd (11.8, 11.2)	2.21, m 2.07, m	2.23, m 2.07, dd (11.8, 11.2)	2.29, m 2.07, m	2.18, m 2.04, m	2.16, m	2.16, m	1.98, m 1.55, m	2.07, d (13.1) 1.29, m	2.11, d (13.1) 1.35, m
12	0.88, s	1.01, s	0.88, s	0.83, s	0.88, s	0.86, s	0.86, s	0.94, s	0.84, s	0.89, s
13	1.05, s	1.03, s	1.05, s	0.96, s	0.96, s	1.04, s	1.04, s	0.99, s	0.95, s	1.01, s
14	1.76, s	1.76, s	1.76, s	1.72, s	1.70, s	1.88, s	1.88, s	1.31, s	1.45, s	1.47, s
15	5.10, s 4.99, s	5.05, s 4.94, s	5.10, s 4.99, s	5.17, s 5.14, s	1.49, s	1.20, s	1.20, s	1.64, s	1.63, s	1.68, s
2′	6.26, d (16.2)		5.74, d (12.7)	5.74, d (12.7)	5.79, d (12.7)	6.26, d (16.2)	5.74, d (12.7)	6.26, d (16.2)	5.74, d (12.7)	6.28, d (16.2)
3′	7.62, d (16.2)		6.82, d (12.7)	6.82, d (12.7)	6.84, d (12.7)	7.62, d (16.2)	6.82, d (12.7)	7.62, d (16.2)	6.82, d (12.7)	7.64, d (16.2)
5′/9′	7.42, d (8.4)		7.64, d (8.6)	7.64, d (8.6)	7.59, d (8.6)	7.42, d (8.4)	7.64, d (8.6)	7.42, d (8.4)	7.64, d (8.6)	7.46, d (8.4)
6′/8′	6.83, d (8.4)		6.81, d (8.6)	6.81, d (8.6)	6.76, d (8.6)	6.83, d (8.4)	6.81, d (8.6)	6.83, d (8.4)	6.81, d (8.6)	6.86, d (8.4)

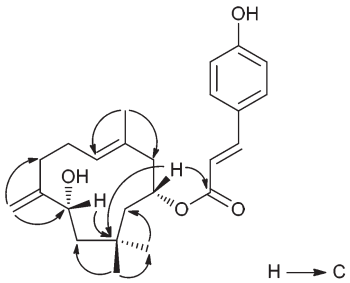


Fig. 2 Key HMBC correlations of **1**.

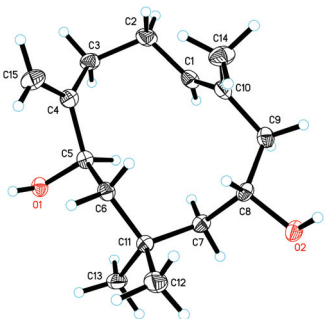


Fig. 3 Perspective ORTEP representation of **1a**.

Consequently, compound **4** was identified as (1*E*,5*R*,8*R*)-8-*O*-[(*Z*)-*p*-coumaroyl]-5-hydroperoxyhumula-1(10),4(15)-dien-8-ol.

From the ESI-HRMS data, compounds **5** and **6** were determined to have the same molecular formula of $C_{24}H_{32}O_3$. Their NMR data (Tables 1 and 2) suggested that they were also a pair of C-2′ configurational isomers. A comparison of the MS and NMR data with literature data[11,14] disclosed **5** as the known 8-*O*-(*p*-coumaroyl)-1(10)*E*,4(5)*E*-humuladien-8-ol with the absolute configuration pending. The sesquiterpenoid skeleton of **5** differed from that of compounds **1–4** in the location of the double bond, which was situated at C-4(5) rather than at C-4(15).

The absolute C-8 configuration of **5** was assumed to be *R* on the basis of biogenesis reasoning, and further confirmed by the Mosher method. Compound **5** was first transformed to alcohol **5a**, and then **5a** was separately treated with (*R*)- and (*S*)-MTPA chloride. From the value differences ($\Delta\delta_{S-R}$) of the key protons (Fig. 4) the absolute configuration of C-8 was designated as *R*, consistent with that of compounds **1–4**. Accordingly, compound **5** was assigned as (1*E*,5*E*,8*R*)-8-*O*-[(*E*)-*p*-coumaroyl]humula-1(10),4(5)-dien-8-ol, and **6** was thereby

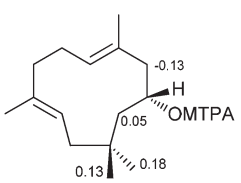


Fig. 4 $\Delta\delta_{S-R}$ values for the MTPA esters of **5a**.

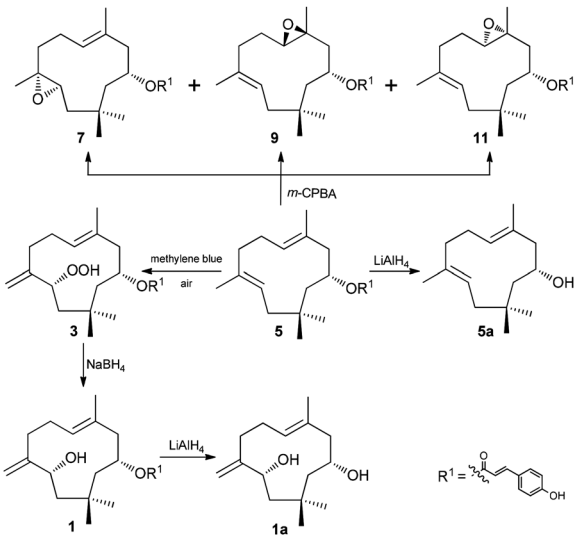
named (1*E*,5*E*,8*R*)-8-*O*-[(*Z*)-*p*-coumaroyl]humula-1(10),4(5)-dien-8-ol.

Compounds **7** and **8** had the same molecular formula of $C_{24}H_{32}O_4$, corresponding to nine degrees of unsaturation. These two compounds were identified by ^{1}H and ^{13}C NMR data (Tables 1 and 2) as a pair of C-2′ configurational isomers. Their NMR spectra showed two oxygenated carbon resonances [**7**: δ_C 60.8 (s), 62.1, (d); **8**: δ_C 60.7 (s), 62.0 (d)] instead of the olefinic C-4 (δ_C 134.6) and C-5 (δ_C 124.3) resonances of **5**[11] which, in combination with the proton signal of one oxygenated C–H (**7**: δ_H 2.60; **8**: δ_H 2.59), revealed the presence of an epoxy group located at C-4 and C-5. The HMBC correlation between H-5 and C-11 further confirmed this deduction. Accordingly, compounds **7** and **8** were identified as the respective epoxidation products of **5** and **6**.

The relative configuration of **7** was tentatively established on the basis of ROESY experiments. The crosspeak of H-8/H-12 indicated that these protons were on the same face, while H-5/H-13 were on the other face on the basis of their crosspeak. The configuration of C-8 would be *R* due to biosynthetic reasoning and, consequently, C-4 and C-5 were both assigned the *R* configuration. Thus, compound **7** was identified as (1*E*,4*R*,5*R*,8*R*)-8-*O*-[(*E*)-*p*-coumaroyl]-4,5-epoxyhumula-1(10)-en-8-ol, and compound **8** was then deduced as being (1*E*,4*R*,5*R*,8*R*)-8-*O*-[(*Z*)-*p*-coumaroyl]-4,5-epoxyhumula-1(10)-en-8-ol.

The molecular formula of **9** was determined to be $C_{24}H_{32}O_4$ from the ESI-HRMS data. A detailed analysis of its ^{1}H and ^{13}C NMR data (Tables 1 and 2) revealed a humulane-type sesquiterpenoid skeleton with an *E*-*p*-coumaroyl substituent. The signals at δ_H 3.12 (d, J = 10.9 Hz), δ_C 62.4 (d), and δ_C 59.2 (s) suggested the presence of an epoxy group, while those at δ_H 5.46, δ_C 123.7 (d), and δ_C 134.2 (s) indicated a trisubstituted double bond. These data suggested that **9** was also an epoxidation product, but different from epoxides **7** and **8**. HMBC correlations from H-14 (δ_H 1.31) to C-9 (δ_C 45.3) and from H-9 (δ_H 1.55, 1.98) to C-1 (δ_C 62.4), C-10 (δ_C 59.2), and C-8 (δ_C 72.0) revealed that the epoxy group was situated at C-1 and C-10, while the HMBC correlations from H-6 (δ_H 2.08, 2.15) to C-4 (δ_C 134.2), C-5 (δ_C 123.7), and C-11 (δ_C 33.6) proved the location of the double bond at C-4 and C-5. The ROESY correlations of H-8/H-1 and H-8/H-5 suggested that these three protons were in the same orientation. Thus, the absolute configuration was assigned as 1*S*,8*R*,10*S*; finally, compound **9** was named as (1*S*,4*E*,8*R*,10*S*)-8-*O*-[(*E*)-*p*-coumaroyl]-1,10-epoxyhumula-4(5)-en-8-ol.

Compounds **10** and **11** had the same molecular formula of $C_{24}H_{32}O_4$, as determined from the respective ESI-HRMS data. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) showed that **10** and **11** were a pair of C-2′ configurational isomers. Similar to **9**, the signals for **10** at δ_H 2.70 (d, J = 10.9 Hz), δ_C 63.2 (d), and δ_C 59.9 (s) indicated the presence of an epoxy group, while those at δ_H 5.11 (d, J = 10.2 Hz), δ_C 123.3 (d), and δ_C 134.0 (s) suggested a double bond in the sesquiterpenoid unit. The double bond was finally placed at C-4 and C-5 on the basis of the key HMBC correlations from H-6 to C-4, 5, and 11 and from H-8 to C-10 and 11. Thus, compound **10** was deduced to have the same planar structure as **9**. In the ROESY spectrum, the observed correlations of H-8/H-12(14) and H-5/H-1(13) clearly suggested that H-1 and H-8 were not on the same face; thus, the absolute configuration of both C-1 and C-10 was proposed as *R* on the basis of the *R*-configured C-8 arising from the biogenesis. Therefore, **10** was established as (1*R*,4*E*,8*R*,10*R*)-8-*O*-[(*Z*)-*p*-coumaroyl]-1,10-epoxyhumula-4(5)-en-8-ol. Consequently, **11** was identified as (1*R*,4*E*,8*R*,10*R*)-8-*O*-[(*E*)-*p*-coumaroyl]-1,10-epoxyhumula-4(5)-en-8-ol.



Scheme 1 Chemical transformations of the isolated compounds.

To further confirm the structures of the isolated compounds, especially the absolute configurations, chemical conversions were designed. As is apparent from Scheme 1, all of the sesquiterpenoids containing an *E*-2′-*p*-coumaroyl group could be derived from **5** (and those containing a *Z*-2′-*p*-coumaroyl group from **6**). Epoxidation of compound **5** with *m*-CPBA in CH_2Cl_2 gave a mixture of compounds **7**, **9**, and **11** in the ratio 87 : 11 : 2, as detected by HPLC. An ene reaction[15,16] was performed to introduce a hydroperoxide group into **5**, yielding **3**. When oxygen was bubbled into a solution of **5** and methylene blue in acetonitrile, the reaction occurred so fast that both double bonds in **5** were peroxidated. With the use of air as the oxygen source, however, compound **3** was successfully obtained as the only oxidative product. Compound **3** was further reduced, affording the relevant hydroxy derivative **1**, the absolute configuration of which was fully confirmed by chemical transformation and single-crystal X-ray diffraction analysis of **1a**.

Conclusions

In summary, 11 humulane-type sesquiterpenoids, including nine new derivatives, were identified from extracts of *P. cavaleriei*. For the first time, the absolute configuration of this uncommon type of sesquiterpene was determined by X-ray

crystallography, as well as by chemical conversions. The ene reaction with air as the oxidizing agent was discovered during the chemical conversions, which gives an indication as to how the allylic hydroperoxide group and related alcohols could be produced in plants. This reaction is more likely to occur in nature than the conversion using oxygen, and might account for the prevalence of these functionalities not only in the isolated sesquiterpenoids but also other natural products. Our findings also suggest that the Urticaceae family might be a rich source of natural sesquiterpenoids, and thus worthy of in-depth investigations.

Experimental section

General experimental procedures

Optical rotations were measured on a Perkin-Elmer 341 polarimeter. Melting points were determined on an XT-4 binocular microscope (Beijing Tech Instrument Co., China) and are not corrected. IR spectra were recorded on a Nicolet Magna FT-IR 750 spectrophotometer using KBr disks. NMR spectra were recorded on Bruker AM-400 and INVOA-600 NMR spectrometers. The chemical shift (δ) values are given in ppm with TMS as the internal standard, and coupling constants (J) are in Hz. ESI-MS and ESI-HRMS data were recorded on Waters 2695-3100 LC-MS and Waters Xevo TOF mass spectrometers. Silica gel (Qingdao Marine Chemical Industrials) was used for flash chromatography. MCI gel CHP20P (75–150 μm, Mitsubishi Chemical Industries, Japan) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden) were used for column chromatography (CC). TLC was carried out on precoated silica gel GF_{254} plates (Yantai Chemical Industrials) and the TLC spots were viewed at 254 nm and visualized with 5% H_2SO_4 in EtOH containing 10 mg mL^{-1} vanillin. Analytical HPLC was performed on a Waters 2690 instrument with a 996 PAD (photodiode array detector) and coupled with an Alltech ELSD 2000 detector. X-ray crystallographic analysis was carried out on a Bruker APEX-II CCD diffractometer with graphite-monochromated Cu Kα radiation (λ = 1.54718 Å).

Plant material

The whole plants of *Pilea cavaleriei* subsp. *crenata* were collected in Guangxi Province, P. R. China in 2009, and identified by Professor Jin-Gui Shen from the Shanghai Institute of Materia Medica. A sample (20090903) was deposited at the Herbarium of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and isolation

The dried and powered plants of *P. cavaleriei* (10 kg) were extracted with 95% EtOH (3 × 40 L, 7 days each) at room temperature. After evaporation of the solvent, the obtained residue was dissolved in water (10 L) and extracted with petroleum ether (PE) (10 L × 3) and ethyl acetate (10 L × 3), successively. The PE fraction was concentrated and extracted with 80% MeOH in water (5 L × 3). The concentrated MeOH extract was subjected to column chromatography (CC) over silica gel; elution with PE–acetone (10 : 1 to 0 : 1) in a stepwise manner gave four fractions (1–4). Fraction 2 was subjected to CC over MCI gel (EtOH–H_2O 70% to 95%) to yield five fractions (2a–2e). Fraction 2a was subjected to CC over Sephadex LH-20 ($CHCl_3$–MeOH 1 : 1) to yield two subfractions (2a1 and 2a2). Fraction 2a1 was purified further by preparative HPLC (CH_3CN–H_2O 40% to 70%) and yielded **7** (19 mg), **8** (9 mg), **9** (7 mg), **10** (5 mg), and **11** (1 mg). Fraction 2d was subjected to CC over Sephadex LH-20 ($CHCl_3$–MeOH 1 : 1) to give two subfractions (2d1 and 2d2). Fraction 2d2 was then subjected to CC over silica gel to afford **5** (2 g) and **6** (300 mg). Fraction 3 was subjected to CC over MCI gel (EtOH–H_2O 70% to 95%) to yield three fractions (3a–3c). Fraction 3a was subjected to CC over Sephadex LH-20 ($CHCl_3$–MeOH 1 : 1) to yield two subfractions (3a1 and 3a2). Compounds **3** (95 mg) and **4** (50 mg) were separated from fraction 3a2 by preparative HPLC. Similarly, compound **11** (3 mg) was purified from fraction 3b2 (obtained from fraction 3b) by preparative HPLC. Fraction 4 was subjected to CC over silica gel ($CHCl_3$–MeOH 1 : 0, 100 : 1, 50 : 1, 0 : 1) to obtain four fractions (4a–4d). Fraction 4b was purified by preparative HPLC, yielding **1** (210 mg) and **2** (90 mg).

Compound characteristics

(1*E*,5*R*,8*R*)-8-*O*-[(*E*)-*p*-coumaroyl]-5-hydroxyhumula-1(10),4-(15)-dien-8-ol (1). $[\alpha]_D^{25}$ +83.0 (*c* 0.1 in MeOH); IR (KBr) ν_{max}/cm^{-1} 3415, 2952, 2857, 1704, 1671, 1631, 1604, 1585, 1515, 1440, 1369, 1328, 1280, 1189, 1168, 1141, 1099, 983, 968, 906, 833, 757, 520; ^1H and ^{13}C NMR: see Tables 1 and 2; ESI-MS *m*/*z* 383 $[M - H]^-$; ESI-HRMS *m*/*z* 383.2238 $[M - H]^-$ (calcd for $C_{24}H_{31}O_4$ 383.2222).

(1*E*,5*R*,8*R*)-8-*O*-[(*Z*)-*p*-coumaroyl]-5-hydroxyhumula-1(10),4-(15)-dien-8-ol (2). White powder; $[\alpha]_D^{25}$ +76.0 (*c* 0.1 in MeOH); IR (KBr) ν_{max}/cm^{-1} 3417, 2929, 2859, 1683, 1631, 1604, 1587, 1513, 1446, 1367, 1326, 1166, 1101, 1049, 970, 908, 833, 516; ^1H and ^{13}C NMR: see Tables 1 and 2; ESI-MS *m*/*z* 383 $[M - H]^-$; ESI-HRMS *m*/*z* 383.2221 $[M - H]^-$ (calcd for $C_{24}H_{31}O_4$ 383.2222).

(1*E*,5*R*,8*R*)-8-*O*-[(*E*)-*p*-coumaroyl]-5-hydroperoxyhumula-1(10),4-(15)-dien-8-ol (3). ^1H NMR ($CDCl_3$) δ 7.60 (1 H, d, *J* = 15.9 Hz), 7.41 (2 H, d, *J* = 8.6 Hz), 6.84 (2 H, d, *J* = 8.6 Hz), 6.27 (1 H, d, *J* = 15.9 Hz), 5.30 (1 H, d, *J* = 6.0 Hz), 5.18 (1 H, s), 5.15 (1 H, s), 4.78 (1 H, d, *J* = 8.8 Hz), 4.25 (1 H, d, *J* = 6.6 Hz), 2.50 (1 H, m), 2.10–2.34 (3 H, m), 2.06 (1 H, dd, *J* = 11.4, 11.4 Hz), 1.89 (1 H, d, *J* = 15.4 Hz), 1.73 (3 H, s), 1.60 (1 H, dd, *J* = 15.6, 5.1 Hz), 1.43 (1 H, d, *J* = 8.6 Hz), 0.98 (3 H, s), 0.82 (3 H, s); ESI-MS *m*/*z* 399 $[M - H]^-$.

(1*E*,5*R*,8*R*)-8-*O*-[(*Z*)-*p*-coumaroyl]-5-hydroperoxyhumula-1(10),4-(15)-dien-8-ol (4). $[\alpha]_D^{25}$ +34.2 (*c* 0.12 in MeOH); IR (KBr) ν_{max}/cm^{-1} 3407, 2954, 2927, 2865, 1681, 1629, 1604, 1587, 1513, 1469, 1446, 1369, 1328, 1276, 1168, 1101, 979, 914, 833, 518; ^1H and ^{13}C NMR: see Tables 1 and 2; ESI-MS *m*/*z* 399 $[M - H]^-$; ESI-HRMS *m*/*z* 399.2162 $[M - H]^-$ (calcd for $C_{24}H_{31}O_5$ 399.2171).

(1*E*,5*E*,8*R*)-8-*O*-[(*E*)-*p*-coumaroyl]humula-1(10),4(5)-dien-8-ol (5). Powder; ^1H NMR ($CDCl_3$) δ 7.60 (1 H, d, *J* = 15.9 Hz), 7.41

(2 H, d, J = 8.6 Hz), 6.84 (2 H, d, J = 8.6 Hz), 6.27 (1 H, d, J = 15.9 Hz), 4.86 (1 H, m), 4.85 (1 H, m), 4.63 (1 H, m), 2.11–2.25 (2 H, m), 2.06–2.14 (2 H, m), 1.73–1.84 (2 H, m), 1.63 (3 H, s), 1.39 (2 H, dd, J = 15.5, 7.9 Hz), 1.46 (3 H, s), 1.07 (3 H, s), 0.90 (3 H, s); ESI-MS m/z 367 $[M - H]^-$.

(1*E*,5*E*,8*R*)-8-*O*-[(*Z*)-*p*-coumaroyl]humula-1(10),4(5)-dien-8-ol (6). $[\alpha]_D^{25}$ +0 (c 0.1 in $CHCl_3$); IR (KBr) ν_{max}/cm^{-1} 3376, 2954, 2921, 2856, 1704, 1675, 1604, 1513, 1440, 1367, 1278, 1166, 981, 831, 518; ^1H and ^{13}C NMR: see Tables 1 and 2; ESI-MS m/z 367 $[M - H]^-$; ESI-HRMS m/z 367.2266 $[M - H]^-$ (calcd for $C_{24}H_{31}O_3$ 367.2273).

(1*E*,4*R*,5*R*,8*R*)-8-*O*-[(*E*)-*p*-coumaroyl]-4,5-epoxyhumula-1(10)-en-8-ol (7). $[\alpha]_D^{25}$ −36.5 (c 0.2 in MeOH); IR (KBr) ν_{max}/cm^{-1} 3386, 3183, 1956, 2927, 2863, 1702, 1631, 1606, 1587, 1513, 1452, 1369, 1326, 1284, 1201, 1164, 1091, 985, 831, 518; ^1H and ^{13}C NMR: see Tables 1 and 2; ESI-MS m/z 383 $[M - H]^-$; ESI-HRMS m/z 383.2196 $[M - H]^-$ (calcd for $C_{24}H_{31}O_4$ 383.2222).

(1*E*,4*R*,5*R*,8*R*)-8-*O*-[(*Z*)-*p*-coumaroyl]-4,5-epoxyhumula-1(10)-en-8-ol (8). $[\alpha]_D^{25}$ −30.5 (c 0.22 in MeOH); IR (KBr) ν_{max}/cm^{-1} 3394, 2956, 2867, 1706, 1683, 1631, 1604, 1587, 1513, 1488, 1369, 1326, 1278, 1166, 985, 943, 833, 516; ^1H and ^{13}C NMR: see Tables 1 and 2; ESI-MS m/z 383 $[M - H]^-$; ESI-HRMS m/z 383.2231 $[M - H]^-$ (calcd for $C_{24}H_{31}O_4$ 383.2222).

(1*S*,4*E*,8*R*,10*S*)-8-*O*-[(*E*)-*p*-coumaroyl]-1,10-epoxyhumula-4(5)-en-8-ol (9). White powder; $[\alpha]_D^{25}$ +16.5 (c 0.1 in MeOH); IR (KBr) ν_{max}/cm^{-1} 3382, 2958, 2927, 2871, 1702, 1631, 1604, 1587, 1513, 1442, 1367, 1330, 1278, 1201, 1166, 1099, 983, 833, 524; ^1H and ^{13}C NMR: see Tables 1 and 2; ESI-MS m/z 383 $[M - H]^-$; ESI-HRMS m/z 383.2212 $[M - H]^-$ (calcd for $C_{24}H_{31}O_4$ 383.2222).

(1*R*,4*E*,8*R*,10*R*)-8-*O*-[(*Z*)-*p*-coumaroyl]-1,10-epoxyhumula-4(5)-en-8-ol (10). $[\alpha]_D^{25}$ +18.5 (c 0.1 in MeOH); IR (KBr) ν_{max}/cm^{-1} 3382, 2958, 2927, 2871, 1702, 1631, 1604, 1587, 1513, 1442, 1367, 1330, 1278, 1201, 1166, 1099, 983, 833, 524; ^1H and ^{13}C NMR: see Tables 1 and 2; ESI-MS m/z 383 $[M - H]^-$; ESI-HRMS m/z 383.2226 $[M - H]^-$ (calcd for $C_{24}H_{31}O_4$ 383.2222).

(1*R*,4*E*,8*R*,10*R*)-8-*O*-[(*E*)-*p*-coumaroyl]-1,10-epoxyhumula-4(5)-en-8-ol (11). $[\alpha]_D^{25}$ +20.0 (c 0.1 in $CHCl_3$); IR (KBr) ν_{max}/cm^{-1} 3349, 3006, 2923, 2854, 1708, 1606, 1587, 1515, 1465, 1278, 1166, 985, 833, 725, 632; ^1H and ^{13}C NMR: see Tables 1 and 2; ESI-MS m/z 383 $[M - H]^-$; ESI-HRMS m/z 383.2219 $[M - H]^-$ (calcd for $C_{24}H_{31}O_4$ 383.2222).

X-ray crystallography of 1a

1a: $C_{16}H_{30}O_3$, M = 270, orthorhombic, colorless, crystal size 0.25 × 0.22 × 0.18 mm, space group $P2_1$, a = 33.5085(13) Å, b = 6.1892(2) Å, c = 8.0369(3) Å, V = 1666.78(10) Å^3, Z = 4, D_{calcd} = 1.078 mg m^{-3}, $F(000)$ = 600, reflections collected 2728, 2643 unique (R_{int} = 0.0373), final R indices for $I > 2\sigma(I)$, R_1 = 0.0516, wR_2 = 0.1472, R indices for all data R_1 = 0.0526, wR_2 = 0.1478, completeness to θ (64.98) 96.7%, maximum transmission 0.9045, minimum transmission 0.8709, absolute structure parameter 0.1(4). The structure was solved by direct methods using the program SHELXS-97 (Sheldrick, 2008) and refined using the full-matrix least-squares method on F^2, GOF = 1.093. The X-ray diffraction data have been deposited with the Cambridge Crystallographic Data Centre (CCDC 925951).

Chemical transformations

Compound 1a. To a solution of natural **1** (40 mg, 0.1 mmol) dissolved in dried THF was added $LiAlH_4$ (5 mg) at 0 °C, and the mixture was stirred at 0 °C for 30 min. Then, MeOH was added to quench the reaction, and the mixture was diluted with water, then extracted with CH_2Cl_2. The combined organic layer was dried (anhydrous Na_2SO_4) and concentrated under reduced pressure to give **1a** (22 mg, 85%); colorless crystals; mp 80–85 °C (MeOH); $[\alpha]_D^{25}$ +28.0 (c 0.1 in MeOH); IR (KBr) ν_{max}/cm^{-1} 3396, 2929, 2852, 1648, 1467, 1448, 1367, 1284, 1161, 1068, 999, 900, 684, 530; ^1H and ^{13}C NMR: see Tables 1 and 2; ESI-MS m/z 221 $[M + H - H_2O]^-$; ESI-HRMS m/z 221.1916 $[M + H - H_2O]^-$ (calcd for $C_{15}H_{25}O$ 221.1905).

Reduction of 3. To a solution of natural **3** (40 mg, 0.1 mmol) dissolved in MeOH (10 mL) was added $NaBH_4$ (5 mg, 0.13 mmol) at room temperature, and the mixture was stirred at 0 °C for 30 min. Then, saturated $NaHCO_3$ (10 mL) and CH_2Cl_2 (20 mL) were added, and the mixture was stirred for an additional 10 min. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. All organic layers were combined and dried (anhydrous Na_2SO_4), then finally concentrated under reduced pressure to give **1** (35 mg 92%).

Compound 5a. To a solution of **5** (100 mg) dissolved in dried THF was added $LiAlH_4$ (15 mg) at 0 °C, and the mixture was stirred at 0 °C for 30 min. Then, MeOH was added to quench the reaction, and the mixture was diluted with water, then extracted with CH_2Cl_2. The combined organic layer was dried (anhydrous Na_2SO_4) and then concentrated under reduced pressure to give **5a** (46 mg 75%); colorless oil; ^1H NMR ($CDCl_3$) δ 4.86 (1 H, m), 4.85 (1 H, m), 3.40 (1 H, m), 2.11–2.25 (2 H, m), 2.06–2.14 (2 H, m), 2.05–2.13 (2 H, m), 1.91–1.99 (2 H, m), 1.01–1.06 (2 H, m), 1.63 (3 H, s), 1.46 (3 H, s), 1.07 (3 H, s), 0.90 (3 H, s).

Preparation of the (*R*)- and (*S*)-MTPA ester derivatives of compound 5a. Compound **5a** (2.0 mg) was added to two separate NMR tubes, and dried under reduced pressure overnight at room temperature. Pyridine-d_5 (0.5 mL) was transferred to each tube, to give a clear solution. (*S*)-(+)-α-Methoxy-α-(trifluoromethyl) phenylacetyl (MTPA) chloride (10 μL) was injected into one NMR tube, and (*R*)-MTPA chloride (10 μL) into the other, under N_2 gas. The NMR tubes, with the reagents, were sealed and stored overnight in a desiccator until the reactions were completed (monitored by ^1H NMR spectroscopy). The ^1H NMR chemical shifts of the (*R*)-MTPA ester and the (*S*)-MTPA ester of **5a** were recorded directly after each reaction. Ambiguous and overlapping signals were not used for the $\Delta\delta_{S-R}$ calculation.

Epoxidation of compound 5 with *m*-CPBA. A solution of compound **5** (183 mg, 0.50 mmol) and *m*-CPBA (90 mg, 0.55 mmol) in CH_2Cl_2 (10 mL) was stirred under ice-bath cooling for 1 h. The reaction mixture was washed successively with Na_2SO_3 and $NaHCO_3$, dried (anhydrous Na_2SO_4), and

concentrated under reduced pressure to give crude residue (200 mg, >100%). The residue was then subjected to preparative HPLC (CH_3CN–H_2O 40% to 70%) to afford compounds **7** (90 mg), **9** (10 mg), and **11** (1.5 mg).

Ene reaction of compound 5. A solution of compound **5** (18 mg, 0.05 mmol) and methylene blue (0.5 mg) in CH_3CN (5 mL) was stirred at room temperature for 6 h. The reaction mixture was washed with saturated NaCl solution, dried (anhydrous Na_2SO_4), and concentrated under reduced pressure to give compound **3** (20 mg 98%).

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