ORIGINAL ARTICLE

Triterpenoids from *Ainsliaea latifolia* and Their Cyclooxyenase-2 (COX-2) Inhibitory Activities
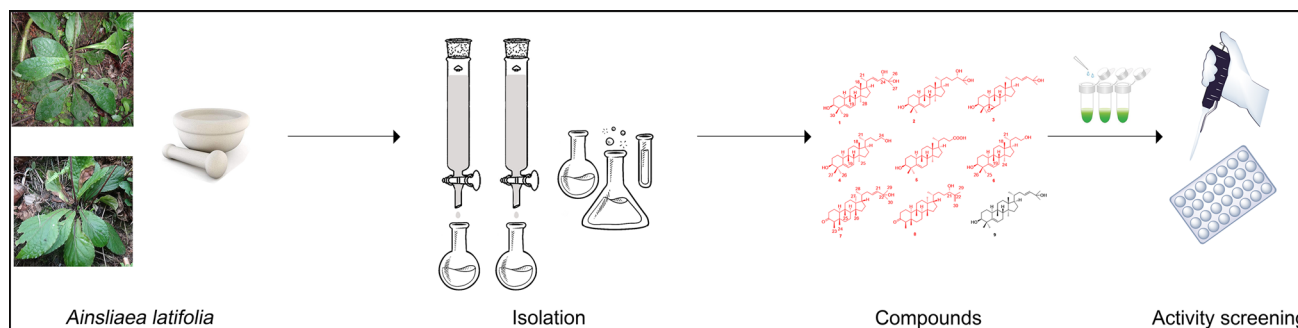
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Abstract

Eight new triterpenoids were isolated from *Ainsliaea latifolia*. The structures of these compounds were elucidated by interpretation of spectroscopic data, including HRESIMS and NMR data. Compounds **4–6** are identified as rare trinorcucurbitane or tetranorcucurbitane triterpenoids. The absolute configurations of compounds **1** and **2** were confirmed by Snatzke's method. All compounds were evaluated for their inhibition against cyclooxyenase-2 (COX-2), in which compound **4** showed significant inhibitory effect against COX-2 with IC_{50} value of 3.98 ± 0.32 μM, comparable to that of positive control NS-398 (IC_{50} 4.14 ± 0.28 μM).

Graphic Abstract

Ainsliaea latifolia   Isolation   Compounds   Activity screening

Dedicated to Professor Han-Dong Sun on the occasion of his 80th birthday.

Wen-Lin Yuan and Xue-Yun Dong have contributed equally to this work.

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1 Introduction

The genus *Ainsliaea* (Compositae), a medicinally important genus in traditional Chinese medicine, comprises about 70 species worldwide, in which most *Ainsliaea* plants are distributed in Southeast Asia. Previous investigations have reported the presence of sesquiterpenoids, sesquiterpene lactone dimers, triterpenoids, steroids and flavonoids in *Ainsliaea* species [1–3]. Some of them exhibited diverse biological activities, including cytotoxic, antiviral, antibacterial and anti-inflammatory activities [4–6].

Ainsliaea latifolia grows mainly in the southwest of China and has long been used as a folk medicine for the treatment of rhumatism, traumatic injuries, edema, stomachache, and

anorexia [7]. In *Ainsliaea* species, sesquiterpenoids are usually considered as characteristic chemical constituents. However, in our study of the chemical constituents from *A. latifolia*, eight new triterpenoids (**1–8**) and one known triterpenoid (**9**) were isolated and identified from the whole plants of *A. latifolia.* Herein, we described the isolation and structural elucidation of compounds **1–8**, as well as their inhibition against cyclooxyenase-2 (COX-2).

2 Results and Discussion

The CHCl$_3$-soluble of the EtOH-H$_2$O (80:20, v/v) extract of *A. latifolia* was purified by repeated column chromatography (CC) over silica gel, Sephadex LH-20, and semi-preparative HPLC to yield eight new and one known compounds. By comparison of their NMR and MS data with the published references, the known compound **9** was then identified as one triterpenoid cucurbita-5,23-diene-3β,25-diol (**9**) [8]. The

structures of eight new triterpenoids were determined by analysis of HRESIMS and NMR spectroscopic data (Fig. 1).

Compound **1** was isolated as white solid. Its molecular formula (C$_{30}$H$_{50}$O$_3$), ascertained via high resolution ESI–MS analysis, indicated six degrees of unsaturation. The ^{1}H NMR spectrum of **1** (Table 1) exhibited signals for three olefinic protons at δ_H 5.59 (2H), 5.42 (1H, m), two oxygenated methine groups at δ_H 3.83 (1H, d, J = 7.1 Hz), 3.47 (1H, brt, J = 2.5 Hz), eight methyl groups (δ_H 1.20, 1.14, 1.13, 1.02, 1.00, 0.92, 0.87, 0.81). The ^{13}C NMR spectrum revealed the presence of thirty carbon signals including four olefinic carbons at δ_C 141.2, 141.3, 125.7 and 121.4, three oxygenated carbons at δ_C 79.7, 76.6 and 72.9, and eight methyl carbons at δ_C 28.0, 27.2, 26.3, 25.4, 23.7, 20.4, 17.8 and 15.7. The other carbon signals were assigned to seven methylenes, four methines, and four quaternary carbons. A comparison of these carbon resonances with those of the related cucurbitane-type triterpenoids suggested that **1** possessed the same cucurbitane skeleton, and the differences between the spectroscopic data of **1** and those of known compound **9** were
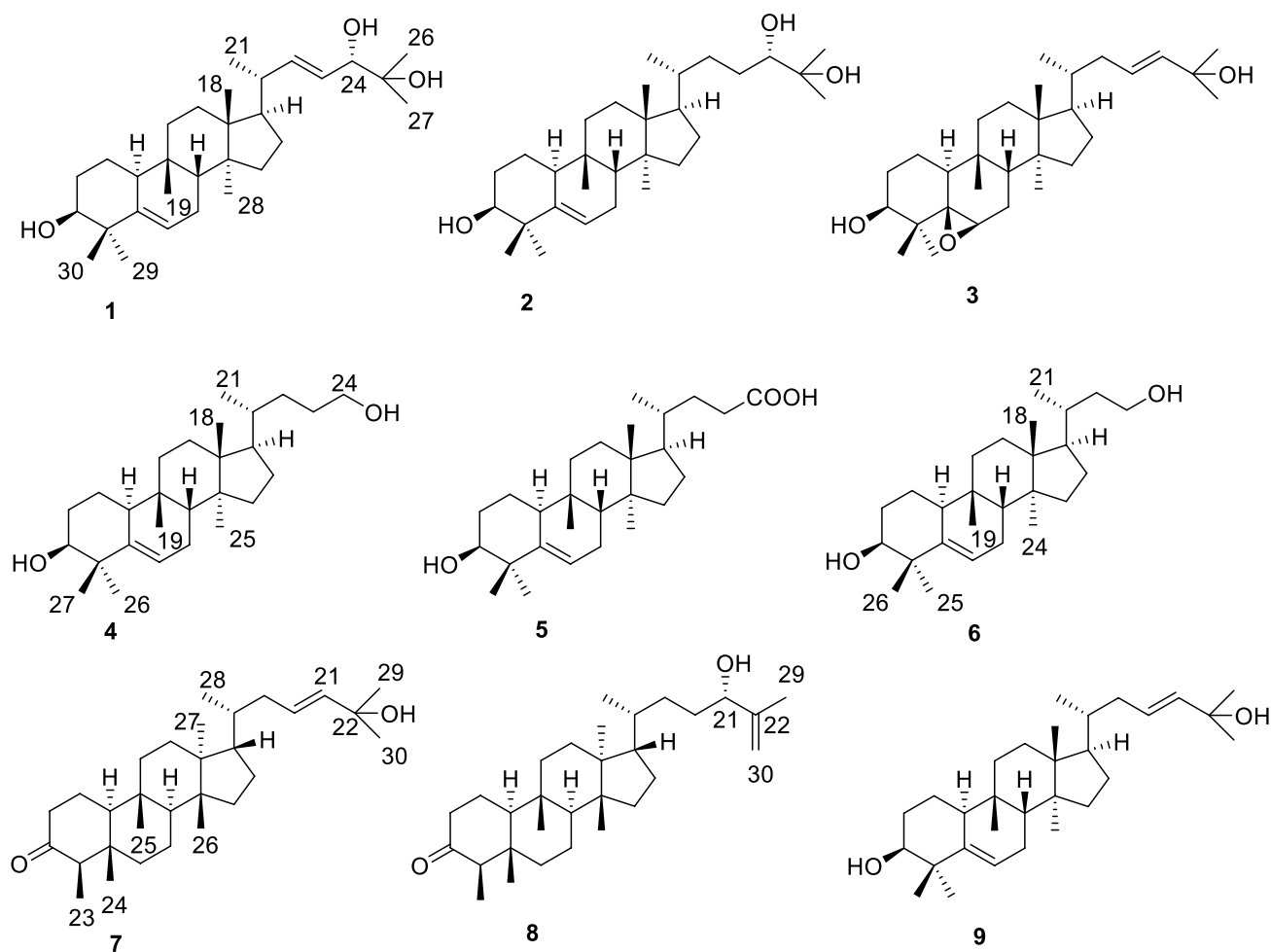

Fig. 1 Chemical structures of **1–9**

Table 1 ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectroscopic data of compounds **1–4** in CDCl$_3$

No.	Compound **1** δ_C	Compound **1** δ_H (*J* in Hz)	Compound **2** δ_C	Compound **2** δ_H (*J* in Hz)	Compound **3** δ_C	Compound **3** δ_H (*J* in Hz)	Compound **4** δ_C	Compound **4** δ_H (*J* in Hz)
1	21.1	1.58, m 1.47, m	21.1	1.58, m 1.47, m	19.9	1.76, m 1.63, m	21.1	1.57, m 1.46, m
2	28.9	1.69, m 1.46, m	28.9	1.69, m 1.46, m	27.5	1.87, m 1.12, m	28.9	1.69, m 1.46, m
3	76.6	3.47, brt (2.5)	76.6	3.47, brt (2.5)	78.5	3.47, s	76.6	3.47, s
4	41.4	–	41.4	–	39.4	–	41.4	–
5	141.2	–	141.2	–	66.8	–	141.2	–
6	121.4	5.59, overlap	121.5	5.59, d (5.9)	53.2	3.16, d (5.8)	121.5	5.59, d (5.7)
7	24.3	2.39, m 1.79, m	24.3	2.39, m 1.79, m	22.7	2.21, m 1.71, m	24.4	2.39, m 1.79, m
8	43.6	1.76, m	43.6	1.76, m	42.4	1.67, m	43.6	1.76, m
9	34.5	–	34.4	–	33.9	–	34.5	–
10	37.8	2.26, d (12.1)	37.8	2.26, d (12.3)	35.2	2.21, m	37.8	2.26, d (12.5)
11	32.3	1.66, m 1.43, m	32.3	1.64, m 1.43, m	33.6	1.63, m 1.32, m	32.3	1.66, m 1.43, m
12	30.4	1.71, m 1.46, m	30.4	1.67, m 1.46, m	30.1	1.64, m 1.46, m	30.4	1.65, m 1.46, m
13	46.3	–	46.2	–	45.8	–	46.2	–
14	49.2	–	49.1	–	49.1	–	49.2	–
15	34.8	1.20, m 1.15, m	34.7	1.20, m 1.14, m	34.6	1.23, m 1.13, m	34.7	1.20, m 1.14, m
16	28.2	1.24, m 1.16, m	27.9	1.24, m 1.16, m	29.7	1.88, m 1.24, m	27.9	1.24, m 1.16, m
17	50.1	1.57, m	50.5	1.57, m	50.4	1.48, m	50.8	1.48, m
18	15.7	0.87, s	15.4	0.85, s	15.3	0.81, s	15.4	0.86, s
19	28.0	0.92, s	28.0	0.91, s	27.1	1.01, s	28.0	0.92, s
20	40.1	2.16, m	36.3	1.45, m	36.2	1.50, m	35.8	1.45, m
21	20.4	1.00, d (6.6)	18.9	0.91, d (6.6)	18.6	0.88, d (5.9)	18.7	0.91, d (5.3)
22	141.3	5.59, overlap	33.6	1.75, m 0.99, m	39.1	2.14, m 1.73, m	29.5	1.05, m 0.92, m
23	125.7	5.42, m	28.6	1.70, m 1.14, m	125.3	5.59, overlap	32.2	1.64, m 1.43, m
24	79.7	3.83, d (7.1)	79.6	3.27, d (9.8)	139.5	5.59, overlap	63.6	3.62, t (6.2)
25	72.9	–	73.2	–	70.7	–	17.8	0.81, s
26	26.3	1.20, s	26.5	1.20, s	29.9	1.31, s	27.2	1.03, s
27	23.7	1.14, s	23.2	1.15, s	30.0	1.31, s	25.5	1.14, s
28	17.8	0.81, s	17.8	0.80, s	20.5	0.85, s		
29	27.2	1.02, s	27.2	1.02, s	24.8	1.12, s		
30	25.4	1.13, s	25.4	1.13, s	19.9	0.88, s		

primarily the observation of an oxymethine and the absence of a methylene. In the ^{1}H–^{1}H COSY spectrum of **1**, two mutual coupling olefinic protons exhibited the correlations with H-20 and the oxygenated methine proton at δ_H 3.83 (Fig. 2), respectively, ascribing a double bond to C-22 and C-23 positions. The HMBC correlation (Fig. 2) of CH$_3$-21 with the olefinic carbon at δ_C 141.3 confirmed the above deduction. Also, the observation of HMBC correlations from CH$_3$-26 and CH$_3$-27 to C-24 (δ_C 79.7) and the oxygenated quaternary carbon at δ_C 72.9 supported the hydroxyl substituents at C-24 and C-25 positions. The absolute configuration of C-24 in **1** was assigned using the Snatzke's method [9, 10]. Metal complex of compound **1** in DMSO gave a significant induced CD spectrum (ICD) (Fig. 4), in which the positive cotton effect observed at 315 nm permitted the assignment of a 24*S* configuration for **1**. The relative configurations of other stereocenters of **1** were established to be identical to those of known compound **9** due to NOESY experiment (Fig. 3). Thus, the structure of compound **1** was identified as cucurbita-5, 22-diene-3β, 24*S*, 25-triol.
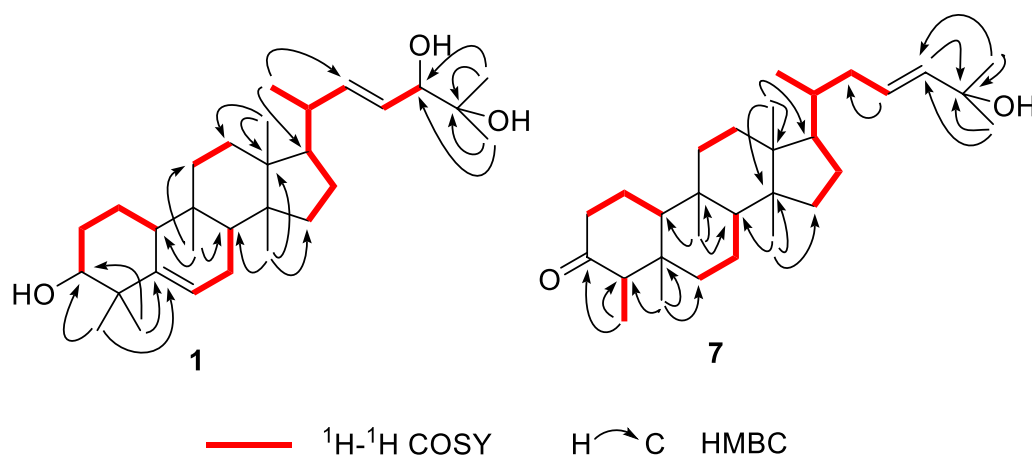
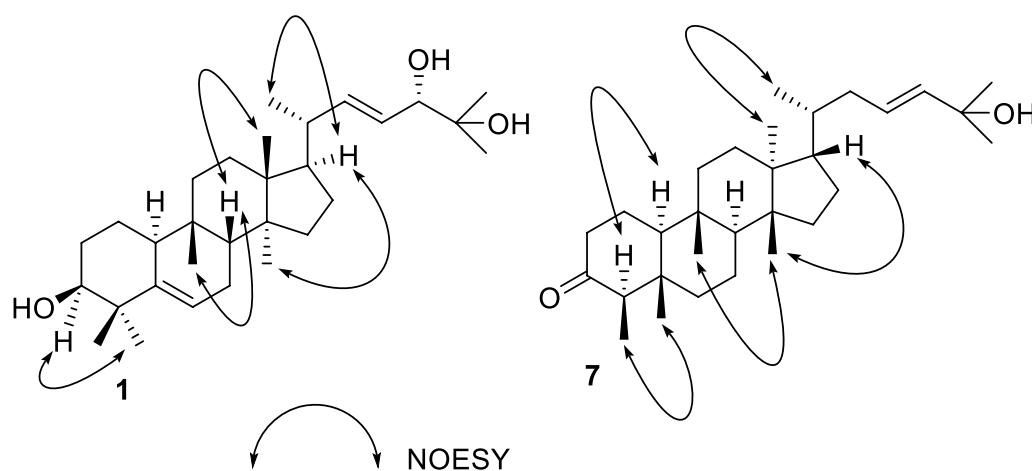**Fig. 2** Selected ^{1}H–^{1}H COSY and HMBC correlations of **1** and **7**

Fig. 3 Key NOESY correlations of **1** and **7**

Compound **2** was obtained as white solid and assigned a molecular formula of $C_{30}H_{52}O_3$ (HRESIMS *m/z* 495.3622 [M + Cl]$^-$, calcd for 495.3610), with two hydrogen atoms more than that of **1** (493.3447 [M + Cl]$^-$). The ^{1}H and ^{13}C NMR spectra (Table 1) of **2** were very similar to **1**, except that two olefinic protons of **1** were replaced by two methylenes in **2**. Therefore, the structure of **2** was determined to be a hydrogenated derivative of **1** at C-22/C-23 double bond. The assignment was confirmed by the ^{1}H–^{1}H COSY correlations of CH$_3$-21/H-20/CH$_2$-22/CH$_2$-23/H-24 and key HMBC correlations of the oxygenated methine proton at δ_H 3.31 (H-24) with C-22 and C-23, and of CH$_3$-26 and CH$_3$-27 with C-24 (δ_C 79.6). Similarly, the absolute configuration of C-24 in **2** was confirmed using the Snatzke's method [9, 10]. The positive Cotton effect observed at 310 nm (Fig. 4) permitted the assignment of a 24*S* configuration for **2**. Thus, the structure of compound **2** was identified as cucurbita-5-ene-3β,24*S*,25-triol.

Compound **3** was isolated as white solid. Its molecular formula ($C_{30}H_{50}O_3$), ascertained via high resolution ESI–MS analysis, indicated six degrees of unsaturation. Detailed analysis of the NMR (Table 1) and MS spectra led to the conclusion that the only difference between **3** and known compound **9** was that there is an epoxide group between C-5 (δ_C 66.8, s) and C-6 (δ_C 53.2, d) in **3** instead of a double bond between C-5 (δ_C 141.2, s) and C-6 (δ_C 121.4, d) in **9**. The epoxide group was elucidated by HMBC correlations of H-1, H-3, H-7, CH$_3$-29 and CH$_3$-30 with C-5, and of H-8 and H-10 with C-6, as well as the ^{1}H–^{1}H COSY correlations of H-6/H-7. The NOESY correlations of H-6/CH$_3$-29 indicated the epoxy ring of **3** was in β-orientation. Thus, the structure of compound **3** was identified as cucurbita-5β,6β-epoxy-23-ene-3β, 25-diol.

Compound **4** was obtained as white solid and assigned a molecular formula of $C_{27}H_{46}O_2$, (HRESIMS *m/z* 403.3594 [M + H]$^+$, calcd for 403.3571), indicating five degrees of
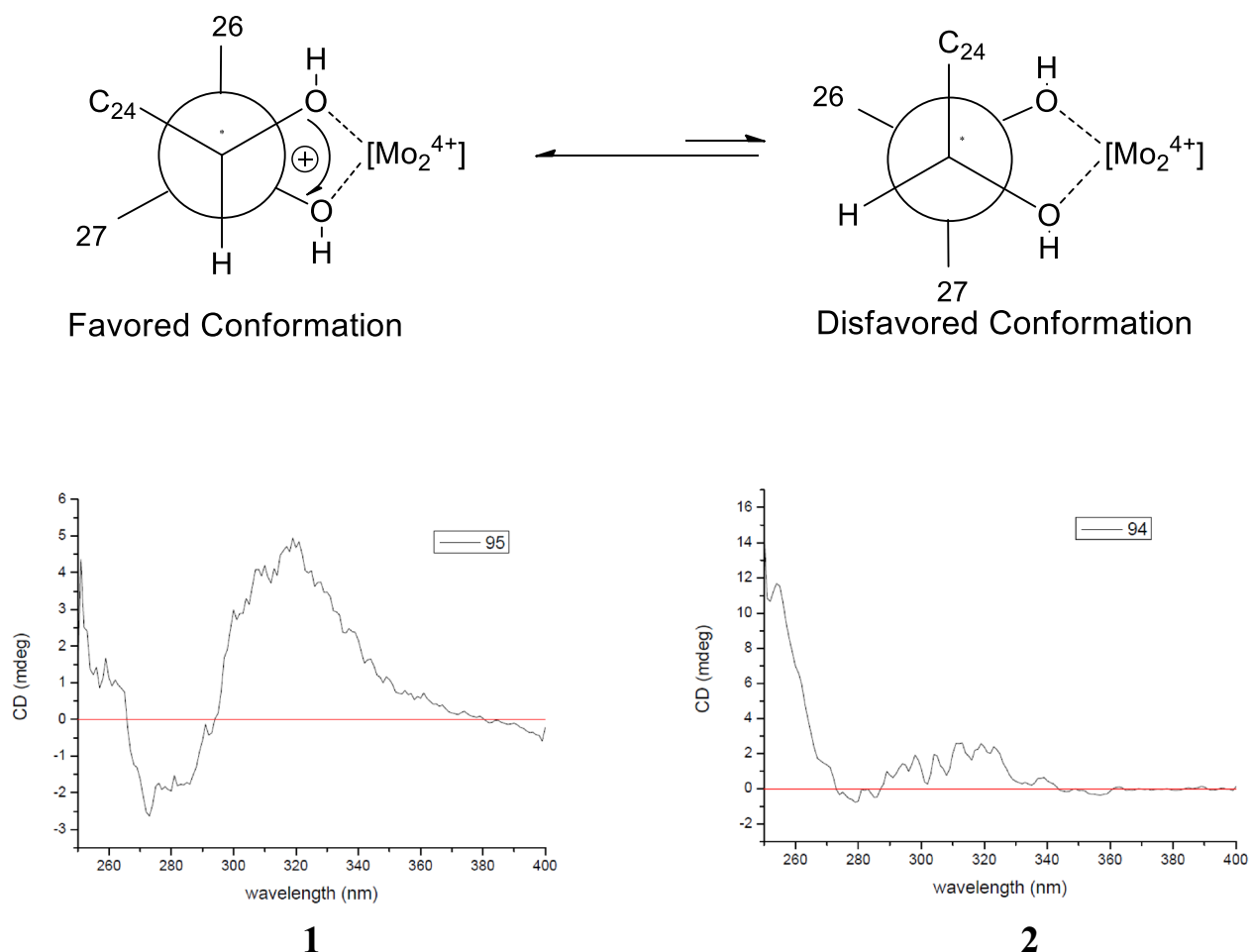
Fig. 4 Conformations and ICD spectra of the Mo_2^{4+} complex of **1** and **2** in DMSO

unsaturation. In the ^{1}H NMR spectrum (Table 1), the signals of five tertiary methyl groups (δ_H 1.14, 1.03, 0.92, 0.86, 0.81) and one secondary methyl group (δ_H 0.91, 3H, d, $J = 5.3$ Hz) were observed. The ^{13}C NMR spectrum of **4** showed signals for 27 carbons due to six methyl groups, two olefinic carbons, ten methylenes (including an oxygenated one), five methines (including an oxygenated one), and four quaternary carbons. Detailed comparison of the ^{13}C NMR spectrum of **4** with that of **2** displayed similarities in rings A–D, except for the absence of the signals for C-25, 26, 27. These evidences revealed that compound **4** is a rare 25,26,27-trinorcucurbitane triterpenoid. This can be confirmed via the ^{1}H–^{1}H COSY correlations of H$_3$-21/H-20/H$_2$-22/H$_2$-23/H$_2$-24. Thus, the structure of compound **4** was identified as 25,26,27-trinorcucurbita-5-ene-3β,24-diol.

Compound **5** was isolated as white solid. Its molecular formula (C$_{27}$H$_{44}$O$_3$), ascertained via high resolution ESI–MS analysis, indicated six degrees of unsaturation. Analysis of the ^{1}H and ^{13}C NMR spectroscopic data of **5** (Table 2) indicated a structural similarity with compound **4**, except

that compound **5** has a carboxyl (δ_C 178.8, C-24) instead of hydroxyl methyl signals in **4**. The deduction was confirmed via the HMBC correlations from H-22, H-23 to the carboxyl carbon (C-24). The relative configurations of **5** were evidenced to be identical to those of **4** by analysis of NOESY spectrum. Thus, the structure of compound **5** was identified as 25,26,27-trinorcucurbita-5-ene-3β-ol-24-acid (Table 3).

Analysis of HRESIMS spectrum ascribed compound **6** to a molecular formula C$_{26}$H$_{44}$O$_2$ due to an adducting ion peak at *m/z* 389.3442 [M + H]$^+$. The NMR data (Table 2) of **6** exhibited one methylene less than those of **4**, which can be confirmed by key ^{1}H–^{1}H COSY correlations of H-21/H-20/H-22/H-23 as well as HMBC correlation from hydroxyl methyl proton at δ_H 3.68 (2H, m) to C-20 (δ_C 33.1). Thus, the structure of compound **6** was identified as a rare 24,25,26,27-tetranorcucurbitane triterpenoid, and named 24,25,26,27-tetranorcucurbita-5-ene-3β,23-diol.

The molecular formula of **7**, C$_{30}$H$_{50}$O$_2$, was determined due to HRESIMS adducting ion peak at *m/z* 443.3904 [M + H]$^+$. The ^{1}H NMR spectroscopic data (Table 2) gave

Table 2 ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectroscopic data of compounds **5-8** in CDCl$_3$

No.	Compound 5 δ_C	Compound 5 δ_H (J in Hz)	Compound 6 δ_C	Compound 6 δ_H (J in Hz)	Compound 7 δ_C	Compound 7 δ_H (J in Hz)	Compound 8 δ_C	Compound 8 δ_H (J in Hz)
1	21.1	1.57, m; 1.46, m	21.1	1.58, m; 1.47, m	22.7	1.95, m; 1.72, m	22.7	1.95, m; 1.72, m
2	28.9	1.69, m; 1.46, m	28.9	1.69, m; 1.46, m	41.5	2.40, m; 2.33, m	41.5	2.42, m; 2.33, m
3	76.6	3.47, brt (2.5)	76.6	3.47, brt (2.5)	213.2	–	213.1	–
4	41.4	–	41.4	–	58.2	2.26, m	58.2	2.26, m
5	141.3	–	141.2	–	42.4	–	42.4	–
6	121.5	5.59, d (5.6)	121.5	5.59, d (5.7)	40.8	1.74, m; 1.34, m	40.8	1.74, m; 1.34, m
7	24.4	2.39, m; 1.79, m	24.4	2.39, m; 1.79, m	20.3	1.53, m; 1.27, m	20.3	1.53, m; 1.27, m
8	43.6	1.76, m	43.6	1.76, m	49.7	1.55, m	49.7	1.55, m
9	34.5	–	34.5	–	37.8	–	37.8	–
10	37.8	2.26, m	37.8	2.26, d (12.1)	59.0	1.58, m	59.0	1.58, m
11	32.3	1.64, m; 1.43, m	32.3	1.66, m; 1.44, m	36.6	1.43, m; 1.39, m	36.7	1.44, m; 1.39, m
12	30.4	1.66, m; 1.48, m	30.4	1.69, m; 1.49, m	30.0	1.71, m; 1.54, m	30.0	1.71, m; 1.54, m
13	46.3	–	46.3	–	46.2	–	46.2	–
14	49.2	–	49.2	–	48.1	–	48.1	–
15	34.7	1.20, m; 1.14, m	34.7	1.46, m; 1.20, m	34.0	2.23, m; 1.20, m	34.0	2.33, m; 1.30, m
16	27.8	1.24, m; 1.16, m	28.1	1.87, m; 1.15, m	27.9	1.89, m; 1.25, m	27.9	1.89, m; 1.24, m
17	50.3	1.48, m	50.8	1.51, m	50.1	1.47, m	50.4	1.46, m
18	15.4	0.86, s	15.3	0.86, s	36.2	1.53, m	35.8	1.50, m
19	28.0	0.92, s	28.0	0.92, s	39.1	2.16, m; 1.75, m	31.9	1.44, m; 0.95, m
20	35.5	1.48, m	33.1	1.57, m	125.4	5.59, overlap	31.5	1.63, m; 1.48, m
21	18.3	0.91, d (5.3)	18.9	0.93, d (5.3)	139.4	5.59, overlap	76.7	4.02, t (6.4)
22	30.9	2.39, m; 2.26, m	39.4	1.72, m; 1.23, m	70.7	–	147.4	–
23	31.1	1.81, m; 1.30, m	61.0	3.68, m (2H)	6.8	0.87, d (6.5)	6.8	0.86, d (6.5)
24	178.8	–	17.8	0.81, s	14.6	0.72, s	14.6	0.72, s
25	17.8	0.81, s	27.2	1.02, s	18.5	0.85, s	18.5	0.85, s
26	27.2	1.03, s	25.5	1.14, s	19.2	0.78, s	19.2	0.78, s
27	25.4	1.14, s			15.8	0.88, s	15.8	0.87, s
28					18.6	0.89, d (6.5)	18.7	0.91, d (5.8)
29					30.0	1.30, s	17.2	1.72, s
30					29.9	1.31, s	111.4	4.93, m; 4.84, m

two olefinic protons at δ_H 5.59 and eight methyls at δ_H 0.87 (d, 6.5 Hz), 0.72 (s), 0.85 (s), 0.78 (s), 0.88 (s), 0.89 (d, 6.5 Hz), 1.30 (s), 1.31 (s). The ^{13}C NMR spectrum revealed the presence of 30 carbon resonances which were sorted into eight methyl carbons, nine methylenes, and seven methine carbons, and six quaternary carbons by DEPT NMR spectrum. Detailed comparison of the NMR data of **7** with those of maytefolin C [11] demonstrated that it possesses the same 18R-D:A-friedoeuphane skeleton, and differs from maytefolin C only at its side chain. The side chain of **7** was determined to be identical to that of known compound **9** by comparison of their ^{1}H and ^{13}C NMR chemical shifts (Table 2). This was further confirmed via the ^{1}H–^{1}H COSY correlations of H-18/H-28, H-18/H-19/H-20 and the key

Table 3 Inhibitory effects of Compounds **1-9** against COX-2 in Vitro

Compounds	COX-2 IC$_{50}$ (μM)	Compounds	COX-2 IC$_{50}$ (μM)
1	>100	**6**	31.02 ± 2.64
2	18.94 ± 1.65	**7**	>100
3	>100	**8**	>100
4	3.98 ± 0.32	**9**	>100
5	19.48 ± 1.87	NS-398	4.14 ± 0.28

HMBC correlations from H-21, CH$_3$-29, CH$_3$-30 to C-22, and from H-20 to C-19 (Fig. 2). The relative configurations of **7** were assigned as shown in Fig. 3 by analysis of the NOESY spectrum (Fig. 3). Thus, the structure of compound **7** was identified as 18*R*-D:A-friedeuph-20-ene-22-ol-3-one.

Compound **8** was obtained as yellow solid, and had the same molecular formula as **7** (C$_{30}$H$_{50}$O$_2$), as ascertained via HRESIMS adducting ion peak at *m/z* 443.3924 [M+H]$^+$. Detailed comparison of the NMR data with those of **7** revealed that **8** possessed a 18*R*-D:A-friedeouphane skeleton as well, differing from **7** only in the positions of the double bond and the oxymethine at the side chain. The HMBC correlations from CH$_3$-29 to two olefinic carbons at δ_C 147.4 and 111.4 disclosed that a terminal double bond was placed at C-22 and C-30 positions. A hydroxyl was substituted at C-21 due to key HMBC correlations of CH$_3$-29 and H-30 with the oxygenated methine carbon at δ_C 76.7. The absolute configuration of C-21 was assigned as *S* on the basis of comparison of the chemical shifts of C-21 (δ_C 76.7) and H-21 (δ_H 4.02, 1H, t, *J* = 6.4 Hz) with those in literature [12]. Thus, the structure of compound **8** was identified as 18*R*-D:A-friedeuph-22(30)-en-21*S*-ol-3-one.

All compounds were evaluated for their COX-2 inhibitory activities with NS-398 as a positive control. The results (Table 3) exhibited that compound **4** had the most potent inhibition against COX-2 with IC$_{50}$ values of 3.98 ± 0.32 μM, while compounds **2**, **5** and **6** showed mild inhibitory effects with IC$_{50}$ values of 18.94 ± 1.65, 19.48 ± 1.87 and 31.02 ± 2.64 μM. Compounds **1**–**6** and **9** share similar or even the same rings A, B, C, D, and the major difference is their side chains. Therefore, it seems that the side chain is the main factor to influence the inhibitions of compounds **1**–**6** and **9** against COX-2.

3 Conclusion

In conclusion, this research led to the isolation of eight new triterpenoids and one known triterpenoid from the *A. latifolia,* in which compounds **4**–**6** are rare trinorcucurbitane or tetranorcucurbitane triterpenoids. It is the first report of cucurbitane-type triterpenoids from the genus *Ainsliaea*.

Interestingly, compound **4** showed potent inhibition against COX-2 with IC$_{50}$ values of 3.98 ± 0.32 μM. These results imply, except for sesquiterpenoids, triterpenoids may be another type of important chemical constituents being responsible for anti-inflammation in *Ainsliaea* species. Therefore, more attention should be paid to structural novel triterpenoids of *Ainsliaea* plants.

4 Experimental Section

4.1 General Experimental Procedures

Optical rotations were measured on a PerkineElmer 341 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance-500 spectrometers. ESI–MS were measured on an Agilent LC/MSD Trap XCT spectrometer, and HRESIMS were performed on an Agilent 6520 Accurate-MS Q-TOF LC/MS system. A preparative column (ZOR-BAX-ODS GSA10250AP1301, C18, 5 μm, 250×10 mm) was used for semi-preparative HPLC (Shimadzu LC-2010A HT). TLC analysis was run on HSGF$_{254}$ silica gel plates (10–40 μm, Yantai, China). Column chromatography (CC) was performed on silica (100–200, 200–300 mesh, Yantai, China), YMC-GEL ODS-A (50 μm, YMC, Japan), Sephadex LH-20 (Amersham Pharmacia Biotech AB, Uppsala, Sweden).

4.2 Plant Material

The dried whole plants of *A. latifolia* were collected from Guiyang city of Guizhou province, PR China in September 2013, and authenticated by Prof. Long Qing-De, Department of Pharmacognosy, School of Pharmacy, Guiyang Medical University. An authentic specimen (No. 20130905) was deposited at the School of Pharmacy, Second Military Medical University.

4.3 Extraction and Isolation

The dried whole plants of *A. latifolia* (15.0 kg) were powdered and extracted with EtOH-H$_2$O (80:20, v/v) twice at room temperature, 48 h each time. The combined EtOH extracts were concentrated *in vacuo* to yield a crude extract (2.0 kg) which was then successfully partitioned with petroleum ether (PE), CHCl$_3$, EtOAc, and MeOH, respectively, The CHCl$_3$ fraction (105 g) was chromatographed on a silica gel column, eluting with gradient PE/EtOAc (100:1; 50:1; 20:1; 10:1; 5:1) to give six fractions (F1: 19.2 g, F2: 5.2 g, F3: 7.3 g, F4: 21.7 g, F5: 7.9 g, F6: 13.1 g). Fraction F2 was subjected to column chromatography (CC) over Sephadex LH-20 (MeOH) and silica gel to give compounds **7** (12.0 mg), **8** (4.2 mg). Fraction F3 was separated

over Sephadex LH-20 (MeOH) followed by semi-preparative HPLC (CH$_3$CN–H$_2$O, 100:0), to yield **1** (3.0 mg), **2** (9.0 mg), and **3** (9.4 mg), respectively. Fraction F4 was subjected to ODS CC, eluted with a MeOH–H$_2$O gradient, to yield 10 subfractions (F4A–F4 J). Subfraction F4B (507 mg) was subjected to CC over Sephadex LH-20 (MeOH) and silica gel to give compounds **4** (4.0 mg), **5** (4.2 mg), **6** (3.2 mg) and **9** (11.7 mg).

4.3.1 Cucurbita-5,22-diene-3β,24*S*,25-triol (**1**)

White solid; $[\alpha]_D^{20}$ + 18.7 (*c* 0.10, CHCl$_3$); UV (MeOH) λ_{max} (log ε) 204 (3.71) nm; For ^{1}H NMR and ^{13}C NMR spectroscopic data, see Table 1; HRESIMS *m/z* 493.3447 [M + Cl]$^-$ (calcd for C$_{30}$H$_{50}$O$_3$, 493.3454).

4.3.2 Cucurbita-5-ene-3β,24*S*,25-triol (**2**)

White solid; $[\alpha]_D^{20}$ + 46.6 (*c* 0.30, CHCl$_3$); UV (MeOH) λ_{max} (log ε) 204 (3.72) nm; For ^{1}H NMR and ^{13}C NMR spectroscopic data, see Table 1; HRESIMS *m/z* 495.3622 [M + Cl]$^-$ (calcd for C$_{30}$H$_{52}$O$_3$, 495.3610).

4.3.3 Cucurbita-5β,6β-epoxy-23-ene-3β,25-diol (**3**)

White solid; $[\alpha]_D^{20}$ + 1.7 (*c* 0.13, CHCl$_3$); UV (MeOH) λ_{max} (log ε) 201 (3.62), 203 (3.69), 231 (3.52) nm; For ^{1}H NMR and ^{13}C NMR spectroscopic data, see Table 1; HRESIMS *m/z* 493.3457 [M + Cl]$^-$ (calcd for C$_{30}$H$_{50}$O$_3$, 493.3454).

4.3.4 Cucurbita-5-ene-3β,24-diol (**4**)

White solid; $[\alpha]_D^{20}$ + 48.0 (*c* 0.31, CHCl$_3$); UV (MeOH) λ_{max} (log ε) 205 (3.73), 207 (3.71) nm; For ^{1}H NMR and ^{13}C NMR spectroscopic data, see Table 1; HRESIMS *m/z* 403.3594 [M + H]$^+$ (calcd for C$_{27}$H$_{46}$O$_2$, 403.3571).

4.3.5 Cucurbita-5-ene-3β-ol-24-acid (**5**)

White solid; $[\alpha]_D^{20}$ + 32.7 (*c* 0.08, CHCl$_3$); UV (MeOH) λ_{max} (log ε) 203 (3.64) nm; For ^{1}H NMR and ^{13}C NMR spectroscopic data, see Table 2; HRESIMS *m/z* 451.2980 [M + Cl]$^-$ (calcd for C$_{27}$H$_{44}$O$_3$, 451.2984).

4.3.6 Cucurbita-5-ene-3β,23-diol (**6**)

White solid; $[\alpha]_D^{20}$ + 9.3 (*c* 0.11, CHCl$_3$); UV (MeOH) λ_{max} (log ε) 205 (3.54) nm; For ^{1}H NMR and ^{13}C NMR spectroscopic data, see Table 2; HRESIMS *m/z* 389.3442 [M + H]$^+$ (calcd for C$_{26}$H$_{44}$O$_2$, 389.3414).

4.3.7 18R-D:A-friedoeuph-20-ene-22-ol-3-one (**7**)

White solid; $[\alpha]_D^{20}$ – 17.4 (*c* 0.37, CHCl$_3$); UV (MeOH) λ_{max} (log ε) 207 (3.18), 231 (3.28) nm; For ^{1}H NMR and ^{13}C NMR spectroscopic data, see Table 2; HRESIMS *m/z* 443.3904 [M + H]$^+$ (calcd for C$_{30}$H$_{50}$O$_2$, 443.3884).

4.3.8 18R-D:A-friedoeuph-22-en-21*S*-ol-3-one (**8**)

White solid; $[\alpha]_D^{20}$ – 37.9 (*c* 0.15, CHCl$_3$); UV (MeOH) λ_{max} (log ε) 201 (3.44), 203 (3.54) nm; For ^{1}H NMR and ^{13}C NMR spectroscopic data, see Table 2; HRESIMS *m/z* 443.3924 [M + H]$^+$ (calcd for C$_{30}$H$_{50}$O$_2$, 443.3884).

4.3.9 Cucurbita-5,23-diene-3*β*,25-diol (**9**)

White solid, C$_{30}$H$_{50}$O$_2$; ^{1}H NMR (500 MHz, CDCl$_3$): δ_H 0.79 (3H, CH$_3$-30), 0.85 (3H, s, CH$_3$-18), 0.87 (3H, d, *J* = 5.8 Hz, CH$_3$-21), 0.91 (3H, s, CH$_3$-19), 1.02 (3H, s, CH$_3$-28), 1.13 (3H, s, CH$_3$-29), 1.30 (2 × CH$_3$, s, CH$_3$-26, 27), 2.26 (1H, d, *J* = 12.1 Hz, H-10), 2.38 (1H, m, H-7), 3.47 (1H, br.t, *J* = 2.5 Hz, H-3), 5.58 (3H, m, H-6, 23, 24); ^{13}C NMR (125 MHz, CDCl$_3$): δ_C 21.1 (t, C-1), 28.9 (t, C-2), 76.6 (d, C-3), 41.4 (s, C-4), 141.2 (s, C-5), 121.4 (d, C-6), 24.3 (t, C-7), 43.6 (d, C-8), 34.5 (s, C-9), 37.8 (d, C-10), 32.3 (t, C-11), 30.3 (t, C-12), 46.3 (s, C-13), 49.2 (s, C-14), 34.8 (t, C-15), 27.8 (t, C-16), 50.1 (d, C-17), 15.4 (q, C-18), 28.0 (q, C-19), 36.2 (d, C-20), 18.7 (q, C-21), 39.1 (t, C-22), 125.5 (d, C-23), 139.4 (d, C-24), 70.7 (s, C-25), 29.8 (q, C-26), 29.9 (q, C-27), 17.8 (q, C-30), 27.2 (q, C-28), 25.4 (q, C-29); ESI–MS: *m/z* 465 [M + Na]$^+$ (positive), 441 [M − H]$^-$ (negative).

4.4 Determination of the Absolute Configuration of C-24 in Compounds 1 and 2

According to the published literature [9, 10], a mixture of compound **1** (1.1 mg) and Mo$_2$(OAc)$_4$ (1.2 mg) was prepared for CD measurement. The mixture was kept for 30 min to form a stable chiral metal complex, the CD spectrum of which was then recorded. The observed sign of the diagnostic ICD (induced CD spectrum) curve at around 315 nm was correlated with the absolute configuration of C-24 in compound **1**. Compound **2** was also dealt with the same method as **1**.

4.5 COX-2 Inhibitory Effect Assay

Cayman's Colorimetric COX Inhibitor Screening Assay provides a convenient method for human recombinant COX-2 to screen isozyme-specific inhibitors. The assay measures the peroxidase component of COXs. The peroxidase activity is assayed colorimetrically by monitoring the appearance

of oxidized N′,N,N,N′-tetramethyl-*p*-phenylenediamine (TMPD) at 590 nm. The COX-2 assay consisted of a 200 μL reaction mixture containing 150 μL assay buffer, 10 μL Heme, 10 μL COX-2, 20 μL Colorimetric Substrate, and 10 μL test solution (1, 5, 10, 20, 80, 100 μmol·L^{-1}). The reactions were initiated by quickly adding 10 μL Arachidonic Acid, then incubating for 2 min at room temperature [13].

Compliance with Ethical Standards

Conflict of interest The authors declare that there are no conflicts of interest.

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