Triterpenoids from *Ainsliaea latifolia* and Their Cyclooxygenase-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 cyclooxygenase-2 (COX-2), in which compound 4 showed significant inhibitory effect against COX-2 with IC50 value of 3.98 ± 0.32 μM, comparable to that of positive control NS-398 (IC50 4.14 ± 0.28 μM).

Graphic Abstract

Keywords *Ainsliaea latifolia* · Triterpenoids · COX-2 · Cucurbitane

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 cyclooxygenase-2 (COX-2).

## 2 Results and Discussion

The CHCl₃-soluble of the EtOH-H₂O (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 (C₃₀H₅₀O₃), ascertained via high resolution ESI–MS analysis, indicated six degrees of unsaturation. The ¹H NMR spectrum of 1 (Table 1) exhibited signals for three olefinic protons at δH 5.59 (2H), 5.42 (1H, m), two oxygenated methine groups at δH 3.83 (1H, d, J = 7.1 Hz), 3.47 (1H, brt, J = 2.5 Hz), eight methyl groups (δH 1.20, 1.14, 1.13, 1.02, 1.00, 0.92, 0.87, 0.81). The ¹³C NMR spectrum revealed the presence of thirty carbon signals including four olefinic carbons at δC 141.2, 141.3, 125.7 and 121.4, three oxygenated carbons at δC 79.7, 76.6 and 72.9, and eight methyl carbons at δC 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](image-url)
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 $\delta_H$ 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 $\delta_C$ 141.3 confirmed the above deduction. Also, the observation of HMBC correlations from CH$_3$-26 and CH$_3$-27 to C-24 ($\delta_C$ 79.7) and the oxygenated quaternary carbon at $\delta_C$ 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-3$\beta$, 24$^S$, 25-triol.

| No. | Compound 1 | Compound 2 | Compound 3 | Compound 4 |
|-----|------------|------------|------------|------------|
|     | $\delta_C$ | $\delta_H$ (J in Hz) | $\delta_C$ | $\delta_H$ (J in Hz) | $\delta_C$ | $\delta_H$ (J in Hz) | $\delta_C$ | $\delta_H$ (J in Hz) |
| 1   | 21.1       | 1.58, m    | 21.1       | 1.58, m    | 19.9      | 1.76, m    | 21.1      | 1.57, m    |
|     | 1.79, m    |            | 1.47, m    |            | 1.63, m   |            | 1.46, m   |            |
| 2   | 28.9       | 1.69, m    | 28.9       | 1.69, m    | 27.5      | 1.87, m    | 28.9      | 1.69, m    |
|     | 1.46, m    |            | 1.46, m    |            | 1.12, 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    | 24.3       | 2.39, m    | 22.7      | 2.21, m    | 24.4      | 2.39, m    |
|     | 1.79, m    |            | 1.79, m    |            | 1.71, 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    | 32.3       | 1.64, m    | 33.6      | 1.63, m    | 32.3      | 1.66, m    |
|     | 1.43, m    |            | 1.43, m    |            | 1.32, m   |            | 1.43, m   |            |
| 12  | 30.4       | 1.71, m    | 30.4       | 1.67, m    | 30.1      | 1.64, m    | 30.4      | 1.65, m    |
|     | 1.46, m    |            | 1.46, m    |            | 1.46, 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    | 34.7       | 1.20, m    | 34.6      | 1.23, m    | 34.7      | 1.20, m    |
|     | 1.14, m    |            | 1.14, m    |            | 1.13, m   |            | 1.14, m   |            |
| 16  | 28.2       | 1.24, m    | 27.9       | 1.24, m    | 29.7      | 1.88, m    | 27.9      | 1.24, m    |
|     | 1.16, m    |            | 1.16, m    |            | 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    | 39.1      | 2.14, m    | 29.5      | 1.05, m    |
|     |            |            | 0.99, m    |            | 1.73, m   |            | 0.92, m   |            |
| 23  | 125.7      | 5.42, m    | 28.6       | 1.70, m    | 125.3     | 5.59, overlap | 32.2      | 1.64, m    |
|     |            |            | 1.14, 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    |           |            |
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 24S configuration for 2. Thus, the structure of compound 2 was identified as cucurbita-5-ene-3β,24S,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
unsaturation. In the $^1$H NMR spectrum (Table 1), the signals of five tertiary methyl groups ($\delta_H$ 1.14, 1.03, 0.92, 0.86, 0.81) and one secondary methyl group ($\delta_H$ 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 H3-21/H-20/H2-22/H2-23/H2-24. Thus, the structure of compound 4 was identified as 25,26,27-trinorcucurbita-5-ene-3$\beta$,24-diol.

Compound 5 was isolated as white solid. Its molecular formula (C27H44O3), 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 ($\delta_C$ 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-3$\beta$-ol-24-acid (Table 3).

Analysis of HRESIMS spectrum ascribed compound 6 to a molecular formula C26H44O2 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/H2-22/H2-23/H2-24. Thus, the structure of compound 4 was identified as 25,26,27-trinorcucurbita-5-ene-3$\beta$,23-diol.

The molecular formula of 7, C30H50O2, was determined due to HRESIMS adducting ion peak at m/z 443.3904 [M + H]$^+$. The $^1$H NMR spectroscopic data (Table 2) gave
two olefinic protons at δH 5.59 and eight methyls at δH 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 13C 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 1H and 13C NMR chemical shifts (Table 2). This was further confirmed via the 1H–1H COSY correlations of H-18/H-28, H-18/H-19/H-20 and the key

| No. | Compound 5 | Compound 6 | Compound 7 | Compound 8 |
|-----|------------|------------|------------|------------|
|     | δC (δH)   | δC (δH)   | δC (δH)   | δC (δH)   |
| 1   | 21.1 1.57, m | 21.1 1.58, m | 22.7 1.95, m | 22.7 1.95, m |
| 2   | 28.9 1.69, m | 28.9 1.69, m | 41.5 2.40, m | 41.5 2.42, 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 | 40.8 1.74, m |
| 7   | 24.4 2.39, m | 24.4 2.39, m | 20.3 1.53, m | 20.3 1.53, 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 | 32.3 1.66, m | 36.6 1.43, m | 36.7 1.44, m |
|     | 1.43, m | 1.44, m | 1.39, m | 1.39, m |
| 12  | 30.4 1.66, m | 30.4 1.69, m | 30.0 1.71, m | 30.0 1.71, m |
|     | 1.48, m | 1.49, m | 1.54, 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 | 34.7 1.46, m | 34.0 2.23, m | 34.0 2.33, m |
|     | 1.14, m | 1.20, m | 1.20, m | 1.30, m |
| 16  | 27.8 1.24, m | 28.1 1.87, m | 27.9 1.89, m | 27.9 1.89, m |
|     | 1.16, m | 1.15, m | 1.25, 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 | 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 | 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 | 39.4 1.72, m | 70.7 – | 147.4 – |
|     | 2.26, m | 1.23, m | – | – |
| 23  | 31.1 1.81, m | 61.0 3.68, m (2H) | 6.8 0.87, d (6.5) | 6.8 0.86, d (6.5) |
|     | 1.30, m | – | 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 | 15.8 0.87, s |
| 28  | – | 18.6 0.89, d (6.5) | 18.7 0.91, d (5.8) | 18.7 0.91, d (5.8) |
| 29  | – | 30.0 1.30, m | 17.2 1.72, s | 17.2 1.72, s |
| 30  | – | 29.9 1.31, s | 111.4 4.93, m | 4.93, m |
|     | – | – | 4.84, m | 4.84, m |
The dried whole plants of A. latifolia (15.0 kg) were powdered and extracted with EtOH-H₂O (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), CHCl₃, EtOAc, and MeOH, respectively. The CHCl₃ fraction (105 g) was chromatographed on a silica gel plate (100–200 mesh, Yantai, China). YMC-GEL ODS-A (50 μm, YMC, Japan), Sephadex LH-20 (Amersham Pharmacia Biotech AB, Uppsala, Sweden). 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. An authentic specimen (No. 20130905) was deposited at the School of Pharmacy, Second Military Medical University.

### 4 Experimental Section

#### 4.1 General Experimental Procedures

Optical rotations were measured on a PerkinElmer 341 polarimeter. ¹H and ¹³C 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 (ZORBAX-ODS GSA10250AP1301, C18, 5 μm, 250 × 10 mm) was used for semi-preparative HPLC (Shimadzu LC-2010A HT). TLC analysis was run on HSGF₂₅₄ 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-H₂O (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), CHCl₃, EtOAc, and MeOH, respectively. The CHCl₃ 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 (CH₃CN–H₂O, 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–H₂O gradient, to yield 10 subfractions (F4A–F4J). 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,24S,25-triol (1)

White solid; [α]D²⁰ + 18.7 (c 0.10, CHCl₃); UV (MeOH) λmax (log ε) 204 (3.71) nm; For 1H NMR and 13C NMR spectroscopic data, see Table 1; HRESIMS m/z 493.3447 [M + Cl]⁻ (calcd for C₃₀H₅₀O₃, 493.3454).

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

White solid; [α]D²⁰ + 46.6 (c 0.30, CHCl₃); UV (MeOH) λmax (log ε) 204 (3.72) nm; For 1H NMR and 13C NMR spectroscopic data, see Table 1; HRESIMS m/z 495.3622 [M + Cl]⁻ (calcd for C₃₀H₅₁O₃, 495.3610).

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

White solid; [α]D²⁰ + 1.7 (c 0.13, CHCl₃); UV (MeOH) λmax (log ε) 201 (3.62), 203 (3.69), 231 (3.52) nm; For 1H NMR and 13C NMR spectroscopic data, see Table 1; HRESIMS m/z 443.3904 [M + H]⁺ (calcd for C₂₇H₄₀O₃, 443.3884).

4.3.4 Cucurbita-5-ene-3β,24-diol (4)

White solid; [α]D²⁰ + 48.0 (c 0.31, CHCl₃); UV (MeOH) λmax (log ε) 205 (3.73), 207 (3.71) nm; For 1H NMR and 13C NMR spectroscopic data, see Table 1; HRESIMS m/z 403.3594 [M + H]⁺ (calcd for C₂₇H₄₇O₂, 403.3571).

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

White solid; [α]D²⁰ + 32.7 (c 0.08, CHCl₃); UV (MeOH) λmax (log ε) 203 (3.64) nm; For 1H NMR and 13C NMR spectroscopic data, see Table 2; HRESIMS m/z 451.2980 [M + Cl]⁻ (calcd for C₂₇H₄₆O₂, 451.2984).

4.3.6 Cucurbita-5-ene-3β,23-diol (6)

White solid; [α]D²⁰ + 9.3 (c 0.11, CHCl₃); UV (MeOH) λmax (log ε) 205 (3.54) nm; For 1H NMR and 13C NMR spectroscopic data, see Table 2; HRESIMS m/z 389.3442 [M + H]⁺ (calcd for C₂₆H₄₄O₂, 389.3414).

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

White solid; [α]D²⁰ − 17.4 (c 0.37, CHCl₃); UV (MeOH) λmax (log ε) 207 (3.18), 231 (3.28) nm; For 1H NMR and 13C NMR spectroscopic data, see Table 2; HRESIMS m/z 493.3404 [M + H]⁺ (calcd for C₃₀H₅₀O₃, 493.3484).

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

White solid; [α]D²⁰ − 37.9 (c 0.15, CHCl₃); UV (MeOH) λmax (log ε) 201 (3.44), 203 (3.54) nm; For 1H NMR and 13C NMR spectroscopic data, see Table 2; HRESIMS m/z 443.3924 [M + H]⁺ (calcd for C₂₇H₄₅O₂, 443.3884).

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

White solid, C₃₀H₅₀O₂; 1H NMR (500 MHz, CDCl₃): δH 0.79 (3H, CH₃-30), 0.85 (3H, s, CH₃-18), 0.87 (3H, d, J = 5.8 Hz, CH₃-21), 0.91 (3H, s, CH₃-19), 1.02 (3H, s, CH₃-28), 1.13 (3H, s, CH₃-29), 1.30 (2 × CH₃, s, CH₃-26, 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), 5.38 (3H, m, H=6, 23, 24); 13C NMR (125 MHz, CDCl₃): δC 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 Mo₂(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 provides a convenient method for human recombinant COX-2 isozyme-specific inhibitors. The assay measures the peroxidase component of COXs. The peroxidase activity is assayed colorimetrically by monitoring the appearance of 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].

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Compliance with Ethical Standards Conflict of interest The authors declare that there are no conflicts of interest.

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References

1. Z.J. Wu, X.K. Xu, H.W. Zeng, Y.H. Shen, J.M. Tian, J. Su, H.L. Li, L. Shan, R.H. Liu, W.D. Zhang, Planta Med. 77, 1545–1550 (2011)
2. Y. Wang, Y.H. Shen, H.Z. Jin, J.J. Fu, X.J. Hu, J.J. Qin, J.H. Liu, M. Chen, S.K. Yan, W.D. Zhang, Org. Lett. 10, 5517–5520 (2008)
3. Z.J. Wu, X.K. Xu, Y.H. Shen, J. Su, J.M. Tian, S. Liang, H.L. Li, R.H. Liu, W.D. Zhang, Org. Lett. 10, 2397–2400 (2008)
4. F. Hilmi, J. Gertsch, P. Brenner, S. Valovic, M. Heinrich, O. Sticher, J. Heilmann, Bioorg. Med. Chem. 11, 3659–3663 (2003)
5. M.T. Lindenneyer, A. Hrenn, C. Kern, V. Castro, R. Murillo, S. Müller, S. Lauf, J. Schulte-Mönting, B. Siedle, I. Merfort, Bioorgan. Med. Chem. 14, 2487–2497 (2006)
6. R.T. Zeng, X.Y. Dong, X. Fang, N. Yang, Z.R. Shi, Z.G. Zhuo, Y.H. Shen, W.D. Zhang, Chem. Biodivers. 14, 1600 (2017)
7. Z.Y. Wu, Flora of Yunnan, vol. 13 (Science Press, Beijing, 2004), p. 642
8. S. Nakano, Y. Fujimoto, Y. Takaishi, C. Osorio, C. Duque, Fitoterapia 75, 609–611 (2004)
9. M. Górecki, E. Jabłońska, A. Kruszewska, A. Suszczyńska, Z. Urbanińczyk-Lipkowska, M. Gerard, J.W. Morzycki, W.J. Szczepak, J. Frelek, J. Org. Chem. 72, 2906–2916 (2007)
10. L. Di Bari, G. Pescitelli, C. Pratelli, D. Pini, P. Salvadori, J. Org. Chem. 66, 4819–4825 (2001)
11. A. Ohsaki, Y. Imai, M. Naruse, S. Ayabe, K. Komiyama, J. Takashima, J. Nat. Prod. 67, 469–471 (2004)
12. W.B. Zhou, G.Z. Zeng, H.M. Xu, W.J. He, N.H. Tan, Molecules 18, 14585–14596 (2013)
13. Z.C. Yang, W.L. Lu, X.Y. Ma, D.D. Song, Phytomedicine 19, 301–305 (2012)

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