Sesquiterpenes from *Ainsliaea fragrans* and Their Inhibitory Activities against Cyclooxygenases-1 and 2

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One new guaiane-type sesquiterpene glycoside, 2′-*O*-*E*-caffeoyl-8α-hydroxy-11α,13-dihydro-3β-*O*-β-D-glucozaluzanin C (1), was isolated from the whole herb of *Ainsliaea fragrans* (Compositae), together with five known sesquiterpene lactones: 8α-hydroxy-11α,13-dihydro-3β-*O*-β-D-glucozaluzanin C (2), 8α-hydroxy-11α,13-dihydrozaluzanin C (3), 3α-hydroxy-11β,13-dihydro-8α-*O*-β-D-glucozaluzanin C (4), 3β-hydroxy-11β,13-dihydro-8α-*O*-β-D-glucozaluzanin C (5), 3β-*O*-β-D-glucozaluzanin C (6). The structures of isolated compounds were established by means of 1D and 2D NMR spectroscopy and chemical methods. All isolates obtained in the present study were evaluated for their inhibitory effects against cyclooxygenases-1 and 2 *in vitro*, and the structure–activity relationships were also discussed.

Key words *Ainslieae fragrans*; Compositae; sesquiterpene; cyclooxygenase-1; cyclooxygenase-2

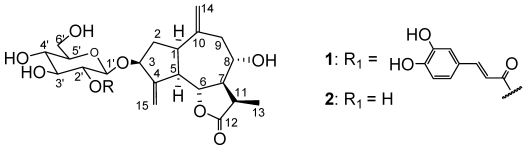
The plant *Ainsliaea fragrans* champ belongs to the Compositae family and grows in the southeast of P. R. China. The whole herb of this plant is widely used in traditional Chinese medicine for the treatment of coughing up blood, jaundice with damp-heat pathogen, edema, and superficial infection.[1] Nowadays, the aqueous extract of *A. fragrans* is widely applied in treating cervicitis, endometritis, and pelvic inflammation, and its clinical effects are significant in China.[2—4] From the aerial parts of *A. fragrans*, sesquiterpene lactones, flavonoids and lignans have been isolated.[5—8] The ethanol extract of *A. fragrans* had been shown to possess significant anti-inflammatory activities on a rat model of cervicitis, which was established by endovaginal injection of latex rubber of oxybenzene in rats, decreasing the prostaglandin E_2 (PGE_2) expressing in an earlier period and regulating the subgroups of T lymphocyte in the rat cervix mucosa.[9]

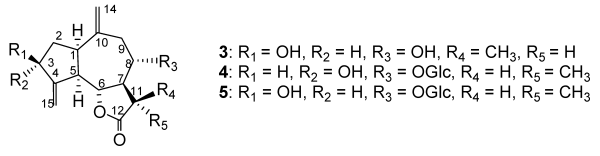
During our search for anti-inflammatory constituents from Chinese medicinal plants, the *n*-BuOH portion from the ethanol extract of *A. fragrans* was found to inhibit the enzyme cyclooxygenases-1 and -2 (COX-1, and -2) *in vitro*, with the concentration exhibiting 50% inhibition (IC_{50}) being 20.28 and 1.18 μg/ml, respectively. Further bioassay-directed fractionation of this extract led to the isolation of six sesquiterpenes. In this paper, we describe the isolation and structure elucidation of one new guaiane-type sesquiterpene glycoside, 2′-*O*-*E*-caffeoyl-8α-hydroxy-11α,13-dihydro-3α-*O*-β-D-glucozaluzanin C (**1**), along with five known sesquiterpenes obtained from this plant. All those isolated were evaluated for their inhibitory effects against both COX-1 and COX-2 *in vitro*, along with clinically used anti-inflammatory drug aspirin.

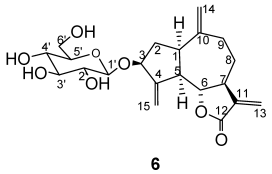
Results and Discussion

The air-dried and powdered herb of of *A. fragrans* was extracted thoroughly with EtOH at room temperature. The ethanol extract of the plant was suspended in water and then successively extracted with petroleum ether (60—90 °C), $CHCl_3$, and *n*-BuOH. The *n*-BuOH portion was chromatographed on silica gel, Sephadex LH-20, and C_{18} repeatedly to afford compounds **1**—**6**. The other five known compounds were subsequently identified as: 8α-hydroxy-11α,13-dihydro-3β-*O*-β-D-glucozaluzanin C (**2**),[8] 8α-hydroxy-11α,13-dihydrozaluzanin C (**3**),[5] 3α-hydroxy-11β,13-dihydro-8α-*O*-β-D-glucozaluzanin C (**4**),[10] 3β-hydroxy-11β,13-dihydro-8α-*O*-β-D-glucozaluzanin C (**5**),[11] 3β-*O*-β-D-glucozaluzanin C (**6**),[12] by comparison of spectral data with literature data values. The purity of these compounds was proved by TLC and HPLC (purity >90% for all compounds).

Compound **1** was obtained as white amorphous powder. The HR-electrospray ionization (ESI)-MS of **1** showed a quasimolecular ion $[M+Na]^+$ at *m*/*z* 611.2125, consistent with a molecular formula $C_{30}H_{36}O_{12}$. The IR spectrum of **1** displayed absorption bands of hydroxyl group at 3425 cm^{-1}, five-membered ring γ-lactone carbonyl at 1753 cm^{-1}, and α,β-unsaturated ester carbonyl group at 1706 cm^{-1}. Further-







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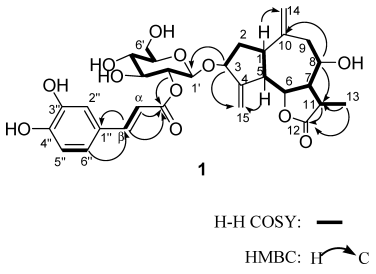

Fig. 1. H–H COSY and Key HMBC Correlations of **1**

more, absorption bands of 1606 and 1524 cm^{-1} revealed the presence of an aromatic skeleton. The ^{13}C and distortionless enhancement by polarization transfer (DEPT) spectra of **1** displayed 30 signals, of which fifteen were assigned to the guaiane-type sesquiterpene aglycone moiety, the remaining fifteen signals corresponding to a hexose sugar residue and a caffeoyl moiety. The ^{1}H-NMR spectrum of **1** showed the presence of one methyl signal [δ 1.09 (3H, d, J=7.7 Hz, H_3-13)], three hydroxylated methane signals [δ 4.64 (1H, m, H-3), 4.04 (1H, t, J=10.2 Hz, H-6), 3.50 (1H, m, H-8)], two pairs of exocyclic olefinic protons [δ 4.90 (1H, br s, H-14a), 5.11 (1H, br s, H-14b), 5.27 (1H, d, J=1.1 Hz, H-15a), 5.32 (1H, d, J=1.1 Hz, H-14b), and an anomeric proton [δ 4.66 (1H, d, J=8.1 Hz, H-1′)]. The ^{1}H-NMR spectrum of **1** also displayed an ABX system assigned to a 1,2,4-trisubstituted aromatic ring [δ 7.02 (1H, d, J=2.0 Hz, H-2″), 6.78 (1H, d, J=8.1 Hz, H-5″), 6.94 (1H, dd, J=1.9, 8.2 Hz, H-6″)], and two trans-olefinic protons [δ 6.24 (1H, d, J=15.9 Hz, H-α), 7.55 (1H, d, J=15.9 Hz, H-β)], suggesting the presence of a caffeoyl moiety. With the aid of ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple quantum correlation (HMQC), and heteronuclear multiple bond connectivity (HMBC) experiments (Fig. 1), all the ^{1}H- and ^{13}C-NMR signals of **1** were assigned as shown in Table 1. Both the ^{1}H- and ^{13}C-NMR signals of **1** were closely related to those of **2** (Table 1), except for the signals due to the caffeoyl moiety. The location of *E*-caffeoyl group through an ester linkage to the C-2′ of the glucose was determined by the HMBC correlations between H-2′ (δ 4.85) and the α,β-unsaturated ester carbonyl carbon (δ 168.3). In addition, the HMBC correlations between H-1′ (δ 4.66) of glucose and C-3 (δ 80.9), as well as H-3 (δ 4.64) and C-1′ (δ 99.4), indicated that the glucose should be attached to C-3 of the aglycone. The relative stereochemistry of **1** was elucidated by means of a nuclear Overhauser effect spectroscopy (NOESY) spectrum, which showed the significant correlations between the methyl group (δ 1.08) and β-positioned H-8 (δ 3.50), as well as between H-6 (δ 4.04) and H-8, indicating that the orientations of the methyl group at C-11 and H-6 were both in β positions. The NOESY experiment also showed a strong cross peak between H-3 (δ 4.64) and α-positioned H-5 (δ 2.85), suggesting that H-3 is α-oriented. Thus, compound **1** was elucidated to a 2′-*O*-*E*-caffeoyl-8α-hydroxy-11α,13-dihydro-3-β-*O*-β-D-glucozaluzanin C.

Compounds **1**—**6** were isolated from the active *n*-BuOH fraction of the ethanol extract of *A. fragrans*. Compounds **2**, **3**, and **6** were the major guaiane-type sesquiterpene lactones of this plant and the other compounds (**1**, **4**, **5**) were present in minor quantities. The *in vitro* inhibitory activity was evaluated against both COX-1 and COX-2 for the isolates obtained in the present study and the results are summarized in Table 2. Compound **3**, a guaianolide sesquiterpene lactone, showed moderate COX-1-inhibiting activity with IC_{50} value of 78.8 μM, comparable to that of representative anti-inflammatory drug aspirin with an IC_{50} value of 77.2 μM. However, five sesquiterpene glycosides (**1**, **2**, **4**—**6**) exhibited no or little COX-1 inhibitory activity, suggesting that the insertion of a glucose moiety at C-3 or C-8 of the guaianolide sesquiterpene aglycone severely reduced the COX-1 inhibitory potential. All tested compounds displayed potent COX-2 inhibitory activities with IC_{50} values ranging from 12.5 to 57.9 μM, in comparison with that of aspirin with an IC_{50}

Table 1. ^{1}H- and ^{13}C-NMR Spectra Data of Compounds **1** and **2**[a)]

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1	2.99 t (9.1)	46.1	3.00 t (9.0)	45.5
	1.98 m	37.8	1.97 ddd (5.1, 7.7, 14.5)	37.9
	2.26 m		2.31 ddd (6.9, 8.8, 14.8)	
3	4.64 m	80.9	4.62 dd (5.6, 6.8)	81.1
4		150.1		150.5
5	2.85 br d (9.4)	52.4	2.86 br d (9.2)	52.0
6	4.04 t (10.2)	80.8	4.39 t (10.3)	80.7
7	2.34 dd (2.1, 8.1)	54.4	2.38 dd (8.1, 10.2)	54.2
8	3.50 m	70.6	3.66 m	70.4
9	2.06 m	46.4	2.16 dd (8.3, 12.6)	45.8
	2.58 dd (4.7, 11.5)		2.72 dd (4.5, 12.5)	
10		144.8		145.4
11	2.72 t (7.8)	39.9	2.79 t (7.8)	40.0
12		181.9		182.0
13	1.09 3H d (7.7)	11.5	1.25 3H d (7.0)	11.3
14	4.90 br s	116.6	4.99 br s	115.9
	5.11 br s		5.11 br s	
15	5.27 d (1.1)	116.2	5.33 d (1.4)	115.2
	5.32 d (1.1)		5.38 d (1.4)	
1′	4.66 d (8.1)	99.4	4.45 d (7.8)	102.1
2′	4.85 m	75.3	3.21 dd (7.9, 8.9)	75.3
3′	3.58 t (8.6)	76.4	3.36 t (8.9)	78.3
4′	3.38 m	72.1	S. O.	71.9
5′	S. O.	78.1	3.26 m	77.9
6′	3.70 dd (5.8, 12.0)	62.8	3.56 m	62.9
	3.91 dd (2.1, 12.0)		3.88 dd (1.9, 12.1)	
1″		127.8		
2″	7.02 d (2.0)	115.4		
3″		149.7		
4″		146.8		
5″	6.78 d (8.1)	116.5		
6″	6.94 dd (1.9, 8.2)	123.0		
α	6.24 d (15.9)	115.3		
β	7.55 d (15.9)	147.3		
C=O		168.3		

a) Measured in CD_3OD. Assignments were established by DEPT, ^{1}H–^{1}H COSY, HMQC and HMBC experiments. *J* values (in Hz) are in parentheses. S. O., signal obscured by the solvent or other signals.

Table 2. Inhibitory Activities of Compounds **1**—**6** against COX-1 and COX-2 *in Vitro*

Compound	IC_{50}±S.D. (μM)	
	COX-1	COX-2
1	>200	32.1±9.3
2	>200	30.6±11.4
3	78.8±18.3	57.9±18.1
4	>200	27.5±6.4
5	>200	28.7±8.4
6	>200	12.5±3.7
Aspirin	77.2±17.6	87.6±20.5

value of 87.6 μM. Among these compounds, the most potent inhibition of the COX-2 enzyme was found for **6** (12.5 μM), which differs from the other tested compounds by possessing a conjugated exomethylene group in its lactone ring (α-methylene-γ-lactone), indicating the α-methylene-γ-lactone function in the molecule of sesquiterpene lactone increased the COX-2 inhibiting activity. These results also postulate that these sesquiterpene lactones may contribute partly to expression of anti-inflammatory activity of *A. fragrans* through COX-2 inhibiting activity.

Experimental

General Experimental Procedures Melting points were determined on an X-4 melting point microscopic apparatus (Beijing, China) and were uncorrected. Optical rotations were measured on a Jasco P-1030 polarimeter. IR spectra were recorded on a Nicolet Impact 410 FT-IR instrument. UV spectra were recorded using a Shimadzu UV-2501 spectrometer. NMR spectra were obtained on Bruker Avance 300 and Bruker Avance 500 spectrometers with CD_3OD as solvent. Electrospray ionization mass spectrometry (ESI-MS) was performed on an Agilent 1100 HPLC/EST instrument. High resolution electrospray ionization mass spectrometry (HR-ESI-MS) was conducted on a Micromass TOF spectrometer. Column chromatography was carried out using silica gel (200—300 mesh, 400 mesh, Qingdao Haiyang Chemical Group Co., Ltd., China), octodecyl silica gel (ODS) (C_{18}, 40—63 μm, Merck), and Sephadex LH-20 (Pharmacia) as a stationary phase. TLC was performed on precoated silica gel 60 F_{254} or RP-18 F_{254} plates (Merck) and visualized under UV light and by spraying with vanillin reagent followed by heating.

Plant Material The whole herb of *Ainsliaea fragrans* was collected from Wuyuan City, Jiangxi Province, P. R. China, in June 2005, and authenticated by Prof. Ming-jian Qing (Dept. of Pharmacognosy, China Pharmaceutical University). A voucher specimen (No. 20050701) was deposited in the herbarium of China Pharmaceutical University, Nanjing.

Extraction and Purification The air-dried and powdered herbs of *A. fragrans* (5.5 kg) were extracted with 95% EtOH (20 l×3) by maceration at room temperature. The EtOH extract was suspended in water and then successively extracted with petroleum ether (60—90 °C), chloroform, and *n*-BuOH. The *n*-BuOH-soluble fraction (160 g) was subjected to silica gel (200—300 mesh, 1000 g) column chromatography using $CHCl_3$–MeOH (95 : 5, 90 : 10, 85 : 15, 70 : 30, each 5 l) as eluents to yield 8 fractions (A—H), based on silica gel TLC [$CHCl_3$–MeOH (9 : 1) and $CHCl_3$–MeOH (85 : 15)] results. Fraction B (1.2 g) was further subjected to silica gel (400 mesh, 50 g) column chromatography using $CHCl_3$–MeOH (95 : 5, 500 ml) as eluents to yield 25 fractions (Frs. B1—B25). Fractions B8—B12 were combined and recrystallized with MeOH to afford **3** (106 mg). Fraction D (29.6 g) was further separated by silica gel (200—300 mesh, 500 g) column eluted with a gradient of $CHCl_3$–MeOH (95 : 5, 90 : 10, each 2 l) to obtain 40 fractions (Frs. D1—D40). Fractions D9—D13 were combined and purified on a C_{18} (40—63 μm, 180 g) low-pressure column using MeOH–H_2O (30 : 70, 45 : 55, each 800 ml) as eluent and further purified by Sephadex LH-20 to afford **1** (35 mg, 300—450 ml), **2** (125 mg, 550—700 ml), and **4** (54 mg, 1150—1500 ml). Fractions D21 and D22 were combined and subjected to silica gel (400 mesh, 50 g) column chromatography using $CHCl_3$–MeOH (88 : 12, 500 ml) as eluent to yield **5** (21 mg, 80—140 ml), and **6** (150 mg, 210—220 ml).

2′-*O*-*E*-Caffeoyl-8α-hydroxy-11α,13-dihydro-3β-*O*-β-D-glucozaluzanin C (**1**): Colourless powder, mp 125—126 °C (MeOH). $[\alpha]_D^{25}$ +35.8° (c=0.06, MeOH). UV λ_{max} (MeOH) nm: 249. IR (KBr) cm^{-1}: 3425, 2923, 1753, 1706, 1606, 1524, 1268, 1071. ESI-MS *m/z*: 589 $[M+H]^+$ ($C_{30}H_{36}O_{12}$). HR-ESI-MS *m/z*: 611.2125 (Calcd for $[C_{30}H_{36}O_{12}+Na]^+$: 611.2098). ^{1}H- and ^{13}C-NMR spectral data see Table 1.

8α-Hydroxy-11α,13-dihydro-3β-*O*-β-D-glucozaluzanin C (**2**): Colourless powder, mp 118—120 °C (MeOH). $[\alpha]_D^{25}$ +41.5° (c=0.05, MeOH). UV λ_{max} (MeOH) nm: 193. IR (KBr) cm^{-1}: 3386, 2929, 2876, 1748, 1649, 1270, 1076. ESI-MS *m/z*: 444 $[M+NH_4]^+$ ($C_{21}H_{30}O_9$). HR-ESI-MS *m/z*: 449.3332 (Cald for $[C_{21}H_{30}O_9+Na]^+$: 449.3259). ^{1}H- and ^{13}C-NMR spectral data see Table 1.

Acid Hydrolysis of 1 A solution of compound **1** (5 mg) in 2 M HCl–MeOH (1 : 1, 5 ml) was refluxed at 90 °C for 3 h, respectively. After being neutralized with NaOH–H_2O, the solution was extracted with EtOAc (5 ml×3). The H_2O layer was concentrated and passed through an Alltech C_{18} SPE cartridge and then separated repeatedly by HPLC [LichroCART NH_2 column (5 μm, 4.6×250 mm); mobile phase: MeCN–H_2O (75 : 25); column temperature: 30 °C; flow rate: 1.0 ml/min; detection: refractive index (RI)] to afford D-glucose [**1**: 1.2 mg, t_R: 8.9 min, $[\alpha]_D^{20}$: +47.2°].[13]

Assay for Inhibition of COX Activity Inhibitory activity of the compounds on COX-1 and COX-2 was assayed by the use of Colorimetric COX (ovine) Inhibitory Screening Assay Kit purchased from Cayman Chemical (Ann. Arbor, MI, U.S.A., Catalog No. 760111) according to the protocol recommended by the supplier. Tested compounds were dissolved in DMSO at a concentration of 1×10^{-2} M as stock solution, and then diluted into appropriate concentrations (200, 100, 30, 10, 3, 1, 0.3 μM) with assay buffer. Results were expressed as mean±S.D. of three independent experiments, each experiment including triplicate sets.

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