ORGANIC
LETTERS

2008
Vol. 10, No. 24
5517−5520

Ainsliatrimers A and B, the First Two Guaianolide Trimers from *Ainsliaea fulvioides*

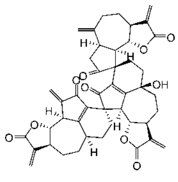
Yan Wang,[†] Yun-Heng Shen,[‡] Hui-Zi Jin,[*,†] Jian-Jun Fu,[†] Xiao-Jia Hu,[†] Jiang-Jiang Qin,[†] Jian-Hua Liu,[†] Ming Chen,[†] Shi-Kai Yan,[†] and Wei-Dong Zhang[*,†,‡]

School of Pharmacy, Shanghai Jiao Tong University, Shanghai 200240, PR China, and
Department of Phytochemistry, Second Military Medical University,
Shanghai 200433, PR China

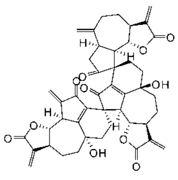
wdzhangy@hotmail.com; kimhz@sjtu.edu.cn

Received September 26, 2008

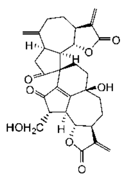
ABSTRACT



Ainsliatrimer A (1)



Ainsliatrimer B (2)



Ainsliadimer B (3)

Ainsliatrimers A (1) and B (2), the first two guaianolide-type sesquiterpene lactone trimers, together with one new structurally related sesquiterpene dimer ainsliadimer B (3), were isolated from the aerial part of *Ainsliaea fulvioides*. Their structures were elucidated by spectroscopic techniques, including X-ray crystal diffraction. Both 1 and 2 showed potent cytotoxicites against LOVO and CEM cell lines.

The genus *Ainsliaea* (Compositae, tribe Mutisieae), a medicinally important genus in traditional Chinese medicine, is mostly distributed in southeast Asia. One of the most abundant groups of secondary metabolites in *Ainsliaea* species are sesquiterpenes, especially guaianolides,[1−3] and the type of guaianolides from this species has been reported to possess diverse biological activities.[4,5] In our continuing search for bioactive compounds from this genus, a novel sesquiterpene lactone dimer with a unique cyclopentane system connecting the two monomeric sesquiterpene lactone units, ainsliadimer A, was isolated from *Ainsliaea macrocephala*.[6] Recently, we investigated another *Ainsliaea* species, *Ainsliaea fulvioides*, which is a medicinal herb growing mainly in southeast of China and has been used as a folk medicine for the treatment of rhumatism, traumatic injuries, edema, stomachache, and anorexia.[7,8] From this plant, we isolated two unprecedented trimeric guaianolides, together with one new guaianolide dimer. Herein, we described the isolation, structure elucidation, and the cytotoxicities of three compounds.

The dried aerial parts of *A. fulvioides* (11.0 kg) were powdered and extracted with 80% ethanol (10 L × 3) at room temperature. After removal of solvent, the extract was

---

[*] To whom correspondence should be addressed. Phone: (86) 21-25070386; (86) 21-34205989. Fax: (86) 21-25070386; (86) 21-34205989.

[†] Shanghai Jiao Tong University.

[‡] Second Military Medical University.

(1) Bohlmamm, F.; Chen, Z. L. *Phytochemistry* **1982**, *21*, 2120–2122.

(2) Adegawa, S.; Miyase, T.; Ueno, A. *Chem. Pharm. Bull* **1987**, *35*, 1479–1485.

(3) Pu, J. X.; Zhao, J. F.; Yang, X. D.; Mei, S. X. *Chin. Chem. Lett.* **2004**, *15*, 1454–1456.

(4) Hilmi, F.; Gertsch, J.; Bremner, P.; Valovic, S.; Heinrich, M. *Bioorg. Med. Chem.* **2003**, *11*, 3659–3663.

(5) Lindenmeyer, M. T.; Hrenn, A.; Kern, C.; Castro, V.; Murillo, R. *Bioorg. Med. Chem.* **2006**, *14*, 2487–2497.

(6) Wu, Z. J.; Xu, X. K.; Shen, Y. H. *Org. Lett.* **2008**, *10*, 2397–2400.

(7) Wu, Z. Y. *Flora of Yunnan*; Science Press: Beijing, China, 2004; Vol. 13, pp 642.

(8) Chinese Herbal Medicine Company. *The Chinese Traditional Medicine Resource Records*; Science Press: Beijing, China, 1994; pp 1241−1243.

10.1021/ol802249z CCC: $40.75 © 2008 American Chemical Society
Published on Web 11/13/2008

successively partitioned with petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH, respectively. The $CHCl_3$ fraction (90.2 g) was chromatographed on a silica gel column, eluting with gradient CH_2Cl_2/MeOH (100:1 → MeOH) to give six fractions. Fraction 3 (19.5 g) was further chromatographed on a silica gel column with gradient petroleum ether/EtOAc (2:1 → 1:1 → EtOAc) to give a subfraction (6.7 g), which was purified by preparative HPLC to yield three compounds, ainsliatrimer A (**1**, 37 mg), ainsliatrimer B (**2**, 12 mg), and ainsliadimer B (**3**, 78 mg).

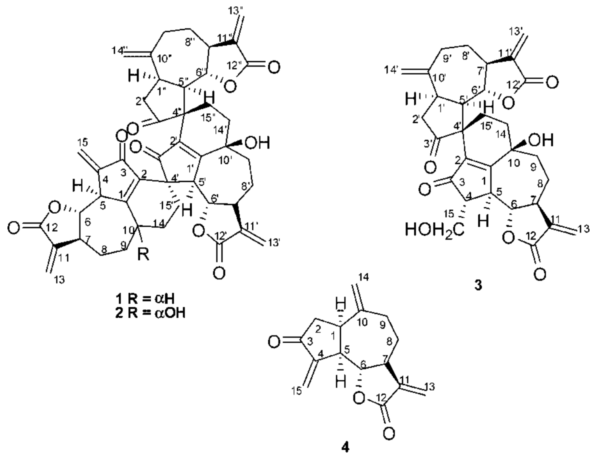


Figure 1. Structures of compounds **1**−**3** and dehydrozaluzanin C (**4**).

Ainsliatrimer A (**1**)[9] was obtained as white powder and had a molecular formula of $C_{45}H_{44}O_{10}$ as determined by HRESIMS at *m/z* 767.2830 $[M + Na]^+$ (calcd 767.2832), indicating 24 degrees of unsaturation. The IR spectrum revealed the presence of hydroxyl (3461 cm^{-1}), γ-lactone (1768 cm^{-1}), and double bonds (1645 cm^{-1}). The ^{13}C and DEPT spectra of **1** indicated 45 carbon signals, including 16 methylenes, 11 methines, and 18 quaternary carbons, of which the typical signals were three carbonyls, three ester carbonyls, 14 olefinic carbons, and four oxygen-bearing carbons. Considering the NMR and MS data, it implied that **1** was a novel sesquiterpene trimer due to its characteristic pair of signals with close chemical shifts in the ^1H and ^{13}C NMR spectra (Table 1).

Detailed analyses of 1D and 2D NMR data of **1** disclosed the ^1H and ^{13}C NMR signals (Table 1) being assigned to three sesquiterpene units A, B, and C (Figure 2), similar to the known sesquiterpene dehydrozaluzanin

Table 1. ^1H and ^{13}C NMR Data for Compounds **1** and **2**[a]

	1		**2**	
no.	δ_H mult (*J* in Hz)	δ_C	δ_H mult (*J* in Hz)	δ_C
1		173.8		172.4
2		138.2		139.1
3		193.9		194.2
4		141.4		141.6
5	3.43 d (10.3)	51.2	3.87 d (10.3)	46.9
6	3.78 t (10.3)	83.2	3.64 t (10.3)	84.0
7	3.21 m	44.0	3.78 m	43.5
8a	1.82 m	24.9	1.80 m	22.9
8b	2.35 m		2.34 m	
9a	2.00 m	28.9	2.05 m	34.2
9b	2.38 m		2.49 m	
10	2.77 m	34.6		72.6
11		139.3		140.2
12		169.4		169.8
13a	5.57 d (3.0)	120.5	5.49 d (3.4)	119.6
13b	6.25 d (3.0)		6.19 d (3.4)	
14a	2.55 m	27.1	2.65 m	38.2
14b	1.64 m		1.93 m	
15a	5.97 s	122.0	5.99 s	123.0
15b	6.26 s		6.30 s	
1′		171.9		172.3
2′		140.3		140.0
3′		207.6		207.3
4′		52.3		51.9
5′	3.19 d (10.0)	58.1	3.17 d (9.6)	58.3
6′	4.36 t (10.0)	80.7	4.35 t (9.6)	80.6
7′	2.65 m	52.2	2.65 m	52.2
8′a	1.99 m	21.2	1.99 m	21.1
8′b	2.15 m		2.08 m	
9′a	1.80 m	36.6	1.78 m	36.6
9′b	1.94 m		2.10 m	
10′		68.3		68.3
11′		138.8		138.6
12′		169.4		169.3
13′a	5.48 d (3.0)	119.3	5.48 d (3.2)	119.5
13′b	6.10 d (3.0)		6.11 d (3.2)	
14′a	2.10 m	36.7	2.09 m	36.6
14′b	1.80 m		1.79 m	
15′a	2.17 m	28.6	2.17 m	31.2
15′b	1.85 m		1.99 m	
1″	3.14 m	39.9	3.13 m	39.9
2″a	3.11 m	44.6	3.07 m	44.5
2″b	2.58 m		2.60 m	
3″		218.8		218.8
4″		50.5		50.5
5″	2.95 m	50.3	2.94 t (9.6)	50.4
6″	4.22 t (9.5)	84.1	4.22 t (9.6)	84.0
7″	2.99 m	43.7	3.00 m	43.7
8″a	1.50 m	31.9	1.52 m	31.9
8″b	2.34 m		2.31 m	
9″a	2.20 m	39.6	2.23 m	39.6
9″b	2.61 m		2.63 m	
10″		150.0		149.9
11″		138.6		138.5
12″		169.4		169.4
13″a	5.59 d (3.0)	121.6	5.59 d (3.2)	121.7
13″b	6.27 d (3.0)		6.28 d (3.2)	
14″a	5.09 s	114.4	5.10 s	114.5
14″b	4.76 s		4.77 s	
15″a	2.30 m	25.8	2.30 m	25.8
15″b	2.06 m		2.05 m	

[a] Data were recorded in $CDCl_3$ at 400 MHz for ^1H and 100 MHz for ^{13}C NMR.

---

(9) Ainsliatrimer A (**1**): white powder; $[\alpha]^{20}_D$ −12.0 (*c* 0.55, $CHCl_3$); ^1H and ^{13}C NMR data, see Table 1; IR (KBr) υ_{max} 3461, 2929, 2867, 1768, 1693, 1645, 1606, 1403, 1259, 1139, 999, 948, 813 cm^{-1}; EIMS *m/z* 745 $[M]^+$, HRESIMS *m/z* 767.2830 (calcd for $C_{45}H_{44}O_{10}Na$, 767.2832).

(10) Chhabra, B. R.; Ahuja, N. M.; Bhullar, M. K.; Kalsi, P. S. *Fitoterapia* **1998**, *69*, 274–275.

(11) Ainsliatrimer B (**2**): white powder; $[\alpha]^{20}_D$ −25.5 (*c* 0.25, $CHCl_3$); ^1H and ^{13}C NMR data, see Table 1; IR (KBr) υ_{max} 3446, 2923, 2850, 1770, 1695, 1646, 1384, 1261, 1139, 997 cm^{-1}; EIMS *m/z* 761 $[M]^+$, HRESIMS *m/z* 783.2778 (calcd for $C_{45}H_{44}O_{11}Na$, 783.2781).

(12) Bohlmann, F.; Ahmed, M.; Jakupovic, J.; King, R. M.; Robinson, H. *Phytochemistry* **1983**, *22*, 191–195.

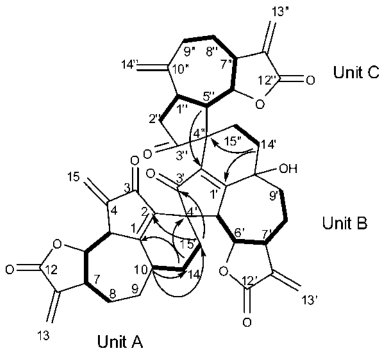


Figure 2. Key $^{1}H-^{1}H$ COSY (—) and HMBC (H to C) (→) correlations of ainsliatrimer A (**1**).

C (**4**),[10] and each sesquiterpene unit included one ester carbonyl, one oxygen-bearing methine, and one ketonic carbonyl. The connectivity of the three units (units A, B, and C) was first achieved by the $^{1}H-^{1}H$ COSY correlations (Figure 2) of H-10 (δ_H 2.77) with H-14α (δ_H 2.55), H-14α (δ_H 2.55) and H-14β (δ_H 1.64) with H-15′β (δ_H 1.85). Further evidence was obtained by analysis of the HMBC spectrum. H-14β (δ_H 1.64) showed the HMBC correlations (Figure 2) to C-1 (δ_C 173.8) and C-4′ (δ_C 52.3); H-15′β (δ_H 1.85) to C-2 (δ_C 138.2); H-10 (δ_H 2.77) to C-14 (δ_C 27.1) and C-15′ (δ_C 28.6), thus establishing the six-membered cyclohexene moiety that connected two sesquiterpene lactone units A and B. Similarly, the HMBC correlations between H-14′β (δ_H 1.80) and C-4″ (δ_C 50.5), and H-5″ (δ_H 2.95) and C-2′ (δ_C 140.2) revealed another six-membered cyclohexene connecting units B and C. The downfield shift of C-10′ (δ_C 68.3), along with chemical shifts of two adjacent carbons C-9′ (δ_C 36.6) and C-14′ (δ_C 36.7), indicated the presence of 10′-hydroxyl group, which was supported by the IR absorption at 3461 cm^{-1}. All proton and carbon resonances were assigned on the basis of analysis of 2D-NMR.

The relative stereochemistry of **1** was determined by the NOESY experiment, in which the correlations between H-5 and H-7/H-10; H-10 and H-5′; H-5′ and H-7′; and H-1″/H-5″ and H-7″ were observed. Thus, H-5, H-7, H-10, H-5′, H-7′, H-1″, H-5″ and H-7″ had the same α-orientation. The large coupling constant between H-6 and H-7 ($J_{H\text{-}6/H\text{-}7} = 10.3$ Hz) suggested a *trans* configuration, and therefore, H-6 must be β-oriented. In addition, H-6′ and H-6″ were also β-orientated as deduced from the large coupling constants ($J_{H\text{-}6'/H\text{-}7'} = 10.0$ Hz, $J_{H\text{-}6''/H\text{-}7''} = 9.5$ Hz, respectively). The relative configurations of H_2-15′ and H_2-15″ were established to be α- and β-oriented by the NOESY correlations of H-5′α with H_2-15′, H-6″β with H_2-15″, respectively. An energy-minimized structure of **1** by MM2 molecular modeling with the selected NOESY correlations was shown in Figure 3.

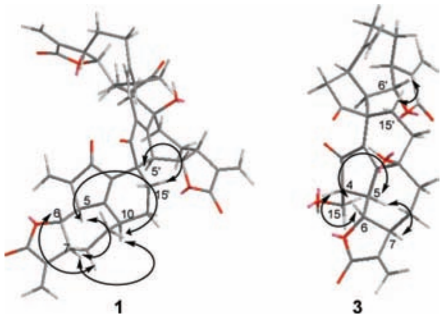


Figure 3. Energy-minimized structures of **1** and **3** with selected NOESY (H↔H) correlations.

Consequently, the structure of **1** was determined for ainsliatrimer A (**1**).

Ainsliatrimer B (**2**)[11] was obtained as white amorphous powder. Its molecular formula was determined to be $C_{45}H_{44}O_{11}$ as deduced from HRESIMS (*m/z* 783.2778 $[M + Na]^+$, calcd for $C_{45}H_{44}O_{11}Na$, 783.2781), indicating 24 degrees of unsaturation. The IR spectrum revealed the presence of hydroxyl (3446 cm^{-1}), γ-lactone (1770 cm^{-1}), and double bonds (1646 cm^{-1}). The ^{13}C NMR spectral data of **2** were very close to those of ainsliatrimer A (**1**) (Table 1). The main differences between **1** and **2** were the chemical shifts of C-10 at δ_C 34.6, C-9 at δ_C 28.9, and C-14 at δ_C 27.1 for **1**, in contrast to 72.6, 34.2, and 38.2 for **2**, respectively. Considering the molecular weights of **2** and **1**, the structure of **2** is only one more oxygen atom than that of **1**. Therefore, it was suggested that **2** was 10-hydroxyl derivative of **1**. The unusual downfield shift of H-7 (δ_H 3.78) could be explained by the proposed 10α-hydroxyl group.[12] Furthermore, all of the correlations supporting the structure of **1** were also observed in the 2D-NMR spectroscopic data of **2**, suggesting that the configuration of **2** was the same as **1**. Accordingly, the structure of **2** was determined as shown in Figure 1.

Ainsliadimer B (**3**),[13] colorless needles (in MeOH), possessed a molecular formula of $C_{30}H_{32}O_8$ as determined by HRESIMS at *m/z* 543.1999 $[M + Na]^+$ (calcd 543.1995),

---

(13) Ainsliadimer B (**3**): colorless needles (in MeOH); mp 159−161 °C; $[\alpha]^{20}_{D}$ −94.0 (*c* 0.11, MeOH); IR (KBr) υ_{max} 3392, 2935, 1766, 1700, 1639, 1405, 1261, 1141, 997, 970, 732 cm^{-1}; EIMS *m/z* 521 $[M]^+$; HRESIMS *m/z* 543.1999 (calcd for $C_{30}H_{32}O_8Na$, 543.1995); ^{1}H NMR (400 MHz $CDCl_3$) δ 1.49 (1H, m, H-8′a), 1.70 (1H, m, H-14b), 1.88 (1H, m, H-9a), 1.95 (1H, m, H-14a), 2.05 (2H, m, H-8a, H-9b), 2.06 (1H, m, H-15′b), 2.10 (1H, m, H-8b), 2.15 (1H, m, H-15′a), 2.21 (1H, m, H-9′a), 2.30 (1H, m, H-8′b), 2.62 (1H, m, H-9′b), 2.66 (1H, m, H-2′b), 2.68 (1H, m, H-4), 2.80(1H, m, H-7), 3.01 (1H, m, H-7′), 3.20 (1H, m, H-5′), 3.22 (1H, m, H-1′), 3.23 (1H, m, H-2′a), 3.33 (1H, dd, $J = 9.8$, 4.3 Hz, H-5), 3.94 (1H, dd, $J = 11.0$, 4.0 Hz, H-15a*), 4.12 (1H, t, $J = 10.5$ Hz, H-6′), 4.35 (1H, t, $J = 9.8$ Hz, H-6), 4.73 (1H, s, H-14′b), 5.09 (1H, s, H-14′a), 5.53 (1H, d, $J = 3.0$ Hz, H-13a), 5.57 (1H, d, $J = 3.0$ Hz, H-13′a), 6.21 (1H, d, $J = 3.0$ Hz, H-13b), 6.26 (1H, d, $J = 3.0$ Hz, H-13′b); ^{13}C NMR (100 MHz $CDCl_3$) δ 21.0 (C-8), 25.8 (C-15′), 32.0 (C-8′), 36.0 (C-9), 36.2 (C-14), 39.5 (C-9′), 40.0 (C-1′), 43.5 (C-7′), 44.7 (C-2′), 49.0 (C-5), 49.6 (C-5′), 51.0 (C-4′), 51.6 (C-7), 53.6 (C-4), 60.6 (C-15), 68.3 (C-10), 82.3 (C-6), 83.8 (C-6′), 114.2 (C-14′), 119.3 (C-13), 121.7 (C-13′), 138.5 (C-11′), 139.3 (C-11), 140.7 (C-2), 150.1 (C-10′), 169.3 (C-12′), 169.7 (C-12), 173.6 (C-1), 207.3 (C-3), 222.0 (C-3′).

(14) Crystallographic data of ainsliadimer B (**3**) have been deposited at the Cambridge Crystallographic Data Centre (deposition no. CCDC 692648). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/deposit.

(15) Denizot, F.; Lang, R. *J. Immunol. Methods* **1986**, *89*, 271–277.

suggesting 17 degrees of unsaturation. The IR spectrum revealed the presence of hydroxyl group (3392 cm^{-1}), γ-lactone (1766 cm^{-1}), and double bonds (1639 cm^{-1}). The ^{13}C NMR and DEPT spectra classified 30 carbon resonances as 11 methylenes, 8 methines, and 11 quaternary carbons. Detailed analysis of the 1D and 2D NMR spectra revealed that **3** was a guaianolide dimer due to the existence of its characteristic pair of carbon signals, which was very similar to known compound dehydrozaluzanin C (**4**). The similar ^{1}H$-^1$H COSY and HMBC correlations of **3** to those of **1** revealed that **3** also had a six-membered cyclohexene bridging two sesquiterpene units. However, **3** lacked exocyclic olefin at C-4 and C-15 and showed the presence of a methane and a hydroxymethyl. The HMBC correlations of H-15 (δ_H 4.10 and 3.94) with C-3 (δ_C 207.3), C-4 (δ_C 53.6), and C-5 (δ_C 49.0) and H-5 (δ_H 3.33) with C-4 (δ_C 53.6) and C-15 (δ_C 60.6) proved the above deduction.

Similar NOESY correlations between H-5 and H-7 and H-5′ and H-7′ placed H-5, H-7, H-5′, and H-7′ in an α-configuration. The large coupling constants ($J_{\text{H-6/H-7}} = 9.8$ Hz and $J_{\text{H-6}'/\text{H-7}'} = 10.5$ Hz) revealed that H-6 and H-6′ were β-oriented. In addition, the NOESY correlations of H-4 and H-6 suggested that H-4 was β-oriented (Figure 3). The relative configulation of H$_2$-15′ was established to be β-oriented by the correlation of H$_2$-15′ with H-6′β.

The structure of **3** was finally confirmed by single-crystal X-ray diffraction and gave an ORTEP drawing as shown in Figure 4.[14]

Three compounds (**1**−**3**) were in vitro evaluated for their antitumor activities against four human cancer cell lines, A549, LOVO, CEM, and MDA-MB-435 (MDA), using MTT assay with DOX (doxorubicin) as positive control.[15] Both **1** and **2** showed potent cytotoxicites against LOVO with IC$_{50}$ (μg/mL) values of 0.18, 0.16 μg/mL and CEM cell lines with IC$_{50}$ (μg/mL) values of 0.16, 0.64 μg/mL, respectively. Moreover, the dimeric sesquiterpene **3** showed modest cytotoxicities with IC$_{50}$ values of 13.84, 1.73 μg/mL against LOVO and CEM cell lines.

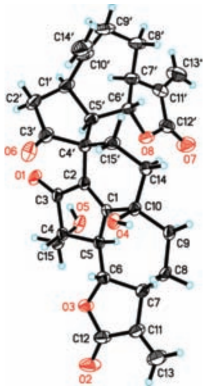


Figure 4. Single-crystal X-ray structure of ainsliadimer B (**3**).

Acknowledgment. The work was supported by program for Changjiang Scholars and Innovative Research Team in University (PCSIRT), NCET Foundation, NSFC (30725045), National 863 Program (2006AA02Z338), China Postdoctoral Science Foundation (20070410711), “973” program of China (2007CB507400), Shanghai Leading Academic Discipline Project (B906) and in part by the Scientific Foundation of Shanghai China (07DZ19728, 06DZ19717, 06DZ19005).

Supporting Information Available: Experimental procedures, physical and spectral data of **1**−**3**, and crystallographic data of ainsliadimer B (**3**). This material is available free of charge via the Internet at http://pubs.acs.org.

OL802249Z