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Ainsliadimer A, A New Sesquiterpene Lactone Dimer with an Unusual Carbon Skeleton from *Ainsliaea macrocephala*

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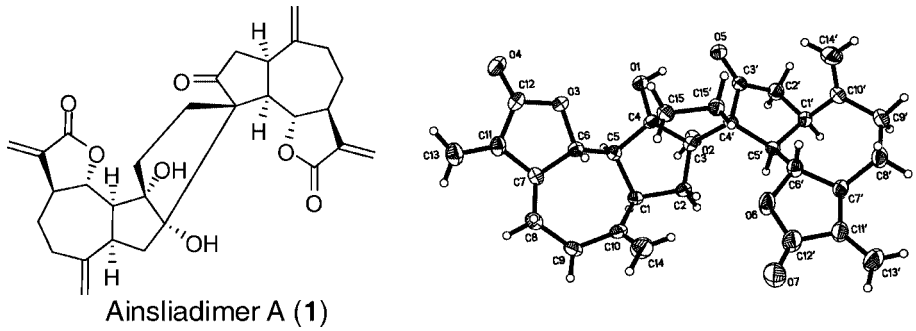
ABSTRACT



Ainsliadimer A (**1**)

A phytochemical investigation of *Ainsliaea macrocephala* led to the isolation of a new dimeric sesquiterpene lactone, ainsliadimer A (1). The structure of 1 was elucidated by spectroscopic analysis, and confirmed by single crystal X-ray diffraction. Ainsliadimer A represents an unusual carbon skeleton with a cyclopentane system connecting the two monomeric sesquiterpene lactone units. This unique molecule exerted potent inhibitory activity against the production of nitric oxide in RAW264.7 stimulated by LPS.

The plants of the Compositae family are well-known to contain structurally diverse and biologically active sesquiterpenoids.[1–3] Within this family, the genus *Ainsliaea* comprises 70 species, 48 of which are indigenous to China.[4] Many *Ainsliaea* species are used in Chinese folk medicine for various indications.[4] Phytochemical studies have indicated that sesquiterpenoids are characteristic constituents of the *Ainsliaea* species.[5–8] In our search for bioactive compounds from this medicinally important genus, a new guaianolide sesquiterpene dimer (**1**) was isolated from *A. macrocephala*, a species native to southwestern China that has never been chemically investigated. In this report, we describe the isolation and structure elucidation of **1** and its inhibitory

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activity against the production of nitric oxide (NO) in RAW264.7 stimulated by LPS.

The whole plants of *A. macrocephala* were collected in Lijiang of Yunnan province, China, and identified by Prof. Li-Shang Xie of Kunming Institute of Botany, Chinese Academy of Sciences. The air-dried whole plants (12 kg) were extracted with 95% EtOH and then successively partitioned with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc-soluble extract was chromatographed over a silica gel column eluting with a gradient $CHCl_3$/MeOH (20:0→4:1) to give three fractions. Fraction 1 was subjected to a silica gel column (petroleum ether/Me_2CO 20:1→5:1) and purified by Sephadex LH-20 ($CHCl_3$/MeOH 1:1) and RP-18 column chromatography (MeOH/H_2O 6:4) to afford ainsliadimer A (**1**, 40 mg, Figure 1).

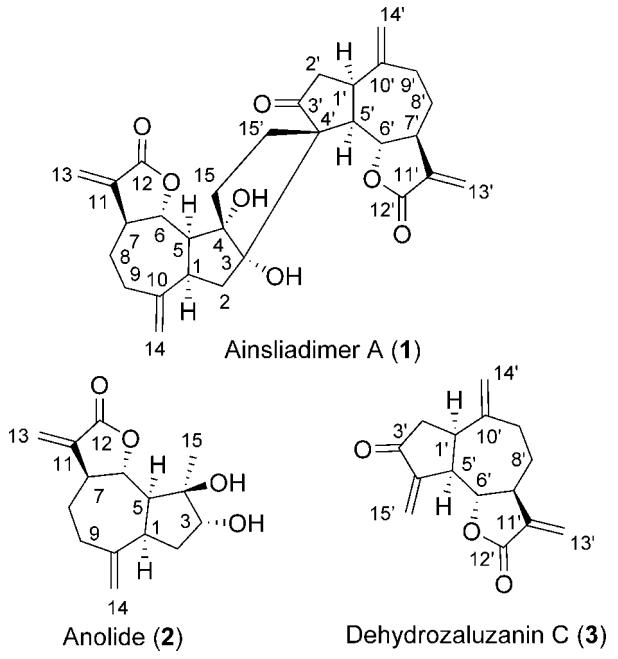

Figure 1. Structures of ainsliadimer A (**1**), anolide (**2**), and dehydrozaluzanin C (**3**).

Ainsliadimer A (**1**)[9] was isolated as colorless prisms. The IR spectrum of **1** suggested the presence of hydroxyl (3471 cm^{-1}), carbonyl (1773 cm^{-1}), and ester carbonyl (1750, 1719 cm^{-1}) groups. Its molecular formula was established as $C_{30}H_{34}O_7$ based on the HRESIMS (negative mode; $[M - H]^-$ *m/z* 505.2170, calcd 505.2226) in conjunction with the ^{13}C NMR spectrum, which requires 14 degrees of unsaturation. The ^{13}C and DEPT NMR spectra of **1** showed 30 carbon resonances due to 12 methylenes, 8 methines, and 10 quaternary carbons, of which the signals of one carbonyl, two ester carbonyls, eight olefinic carbons, and four oxygen-bearing carbons are typical. These NMR and MS data suggested that **1** seemed to be a dimeric sesquiterpene lactone due to its characteristic pair of signals with close chemical shifts in the ^1H and ^{13}C NMR spectra (Table 1).

Table 1. ^1H and ^{13}C NMR Data for Compound **1** in $CDCl_3$

	1 (part **a**)			**1** (part **b**)	
no.	δ_H mult. (*J* in Hz)	δ_C	no.	δ_H mult. (*J* in Hz)	δ_C
1	3.15 (dd, 11.4, 12.0)	41.8	1′	3.04−3.06[a]	50.1
2α	2.38 (dd, 12.0, 13.8)	38.1	2′α	2.91 (dd, 16.8, 9.6)	46.9
2β	1.88−1.92[a]		2′β	2.45 (d, 16.8)	
3		90.5	3′		224.9
4		89.1	4′		62.2
5	2.55 (t, 11.4)	53.1	5′	3.04−3.06[a]	38.9
6	4.07 (dd, 9.0, 11.4)	82.9	6′	4.12 (dd, 8.4, 10.2)	84.0
7	2.72 (m)	49.0	7′	3.04−3.06[a]	43.7
8α	2.22−2.24 (m)	31.3	8′α	2.25−2.30[a]	32.8
8β	1.37 (dq, 6.0, 12.6)		8′β	1.50 (dq, 3.6, 12.6)	
9α	2.67 (m)	37.8	9′α	2.16−2.21[a]	38.9
9β	1.88−1.92[a]		9′β	2.57 (m)	
10		147.5	10′		150.0
11		139.5	11′		138.6
12		170.0	12′		169.0
13a	6.19 (d, 3.0)	119.7	13′a	6.31 (d, 3.0)	122.0
13b	5.46 (d, 3.0)		13′b	5.61 (d, 3.0)	
14a	5.15 (s)	113.8	14′a	4.96 (s)	113.7
14b	5.03 (s)		14′b	4.59 (s)	
15a	2.25−2.30[a]	33.1	15′a	2.05 (dd, 5.4, 14.4)	26.3
15b	2.16−2.21[a]		15′b	2.01 (dd, 7.8, 14.4)	

[a] Signals overlapped.

Careful analysis of the NMR spectra of **1**, including 2D NMR, allowed the ^1H and ^{13}C NMR signals (Table 1) being assigned to two sesquiterpene lactone units designated as parts **a** and **b** (Figure 2). The ^1H and ^{13}C NMR of part **a** gave six methylenes, four methines, and five quaternary carbons, including two exocyclic double bonds, one ester carbonyl, one oxygen-bearing methine, and two oxygen-bearing quaternary carbons, indicating a guaianolide derivative, which is very similar to the known compound anolide (**2**).[10] The differences of the chemical shifts for C-3, C-4, and C-15 between them, together with the HMBC correlations of H_2-2 (δ_H 1.91 and 2.67) with C-3 (δ_C 90.5), C-4 (δ_C 89.1), C-5 (δ_C 53.1), C-10 (δ_C 147.5), and H_2-15 with C-3, C-4, C-5, indicated that part **a** was substituted at C-15 (δ_C 33.1) and C-3. The ^1H and ^{13}C NMR signals of part **b**, with the typical nature of a guaianolide skeleton, showed great similarities to those of known compound dehydrozaluzanin C (**3**) previously reported in the literature,[11] except for the presence of a quaternary carbon (δ_C 62.2) and a methylene (δ_C 26.3) replacing an exocyclic double bond between C-4 and C-15 in dehydrozaluzanin C. The connection between parts **a** and **b** was first indicated by the $^1H-^1H$ COSY correlation between H_2-15 and H_2-15′. Further evidence from the HMBC correlations of H_2-15 with C-4′ (δ_C 62.2), H_2-15′

(9) Ainsliadimer A (**1**): colorless prism; $[\alpha]_{20}^{D}$ +47° (*c* 0.05, $CHCl_3$); IR (KBr) υ_{max} 3471, 2926, 1773, 1751, 1719, 1635,1460, 1262, 989 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 505.2170 $[M - H]^-$ (calcd, 505.2202).

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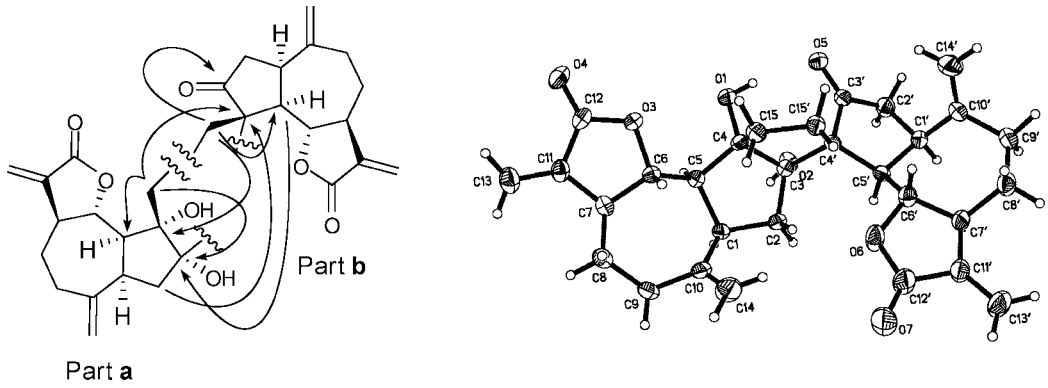
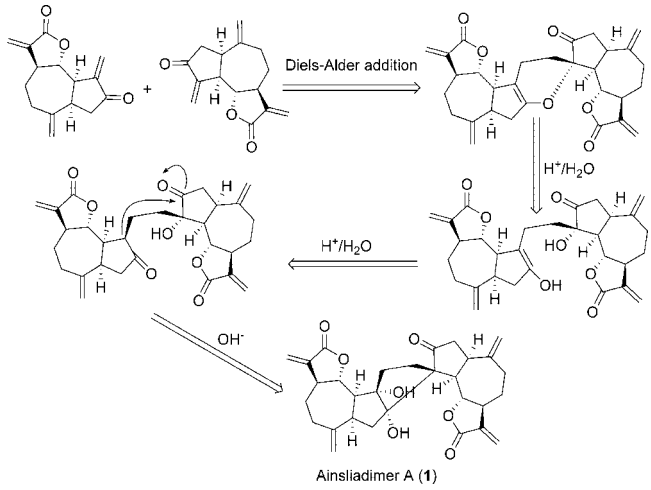


Figure 2. Key HMBC correlations (H→C) and X-ray crystallographic structure of **1**.

(δ_H 2.04) with C-3′ (δ_C 224.9), C-5′ (δ_C 38.9), and C-4, H_2-2 with C-4′, and H-5′ (δ_H 3.05) with C-3 confirmed the connectivity between C-15 and C-15′, and a new C−C bond between the two quaternary carbons C-3 and C-4′. Thus, an unusual cyclopentane system was formed between two sesquiterpene lactone units. The relative configurations of H_2-15 and H_2-15′ were established to be β-oriented by the NOESY correlations of H_2-15 with H-6β, and H_2-15′ with H-6′β. The structure of **1** was finally confirmed by the X-ray diffraction study and an ORTEP drawing is shown in Figure 2.[12] Compound **1** represents an unprecedented carbon skeleton because of the unique cyclopentane system connecting the two sesquiterpene lactone units.

Scheme 1. Plausible Biogenetic Pathway for Ainsliadimer A (**1**)



Numerous dimeric sesquiterpene lactones have been isolated from the family Compositae as well as other plants.[13] They are generally biosynthesized from two monomeric sesquiterpene lactones through a six-membered cyclohexene or 3,4-dihydro-2*H*-pyran ring via regular or hetero Diels−Alder cycloaddition reactions.[14] However, compound **1** represents the first dimeric sesquiterpene lactone with a five-membered cyclopentane ring and is, thus, of particular significance from the perspective of biosynthesis. A plausible biogenetic pathway for ainsliadimer A (**1**) is proposed in Scheme 1. The key step of this biogenetic pathway is Diels−Alder cycloaddition. Recently, the possibility of molecular skeletons of many natural products being constructed by enzymatic Diels−Alder reactions has been attracting increasing interest, and a number of investigations have been carried out to identify Diels−Alderase in the biosynthesis of natural products.[14] Some natural Diels-Alderases, such as solanapyrone synthase,[15] lovastatin nonaketide synthase,[16] and macrophomated synthase,[17] have been isolated and characterized. Therefore, the possible Diels−Alderase in the biosynthesis of ainsliadimer A (**1**) is an interesting topic to be studied

NO plays an important role in the inflammatory process,[18] and an inhibitor of NO release may be considered as a therapeutic agent in the inflammatory diseases.[19] Therefore, compound **1** was tested for the inhibitory activity against the production of NO in RAW264.7 stimulated by lipopolysaccharide (LPS), as described previously.[20] Compound **1** exhibited a remarkable inhibitory effect against the production of NO, with an IC_{50} value of 2.41 μg/mL.

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(12) Crystallographic data of ainsliadimer A (**1**) have been deposited at the Cambridge Crystallographic Data Centre (deposition no. CCDC 680703). Copies of these data can be obtained free of charge via www.ccdc.cam.an.uk/conts/retrieving.html.

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Supporting Information Available: Experimental procedures, 1D and 2D NMR spectra and data, and crystallographic data of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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