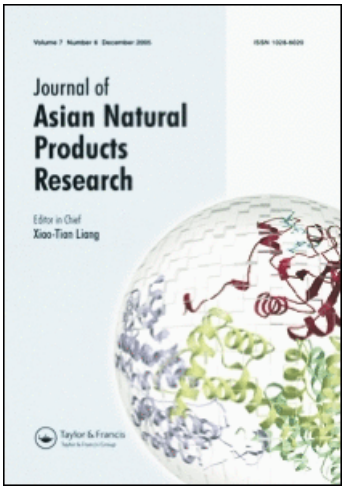

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[a] College of Chinese Traditional Medicine, Guangzhou University of Chinese Medicine, Guangzhou, China [b] Instrumental Analysis of Research Center, Sun Yat-Sen University, Guangzhou, China [c] College of Pharmacy, Nihon University, Chiba, Japan

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Gerardianin A, a new abietane diterpenoid from *Isodon lophanthoides* var. *gerardianus*

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[a]*College of Chinese Traditional Medicine, Guangzhou University of Chinese Medicine, Guangzhou, China;* [b]*Instrumental Analysis of Research Center, Sun Yat-Sen University, Guangzhou, China;* [c]*College of Pharmacy, Nihon University, Chiba, Japan*

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A new abietane diterpenoid, gerardianin A (**1**), along with a known compound 6,7-dehydroroyleanone (**2**), has been isolated from the aerial parts of *Isodon lophanthoides* var. *gerardianus* [Bentham] H. Hara. The structure of **1** was determined on the basis of spectroscopic methods and X-ray single-crystal diffraction analysis.

Keywords: *Isodon lophanthoides* var. *gerardianus*; diterpenoid; NO production inhibitory activity; gerardianin A

1. Introduction

Isodon lophanthoides var. *gerardianus* [Bentham] H. Hara [1], commonly named as *Xihuangcao*, is widely distributed in South and Southeastern Asia. It has been used as a folk medicine in China for the treatment of hepatitis, cholecystitis, dysentery, trauma, enteritis, jaundice, laryngopharyngitis, lepromatous leprosy, and ascariasis [2]. A series of abietane diterpenoids have been obtained from the *Isodon* species[3–5]. Bioassays showed that the EtOAc soluble portion has significant liver-protecting activity in liver injury mice induced by concanavalin A (ConA) [6]. Further study on the EtOAc soluble portion led to the isolation of a new abietane diterpenoid, gerardianin A (**1**), along with a known compound 6,7-dehydroroyleanone (**2**) [7]. Compounds **1** and **2** showed weak nitric oxide (NO) production inhibitory activity with IC_{50} values of 20.5 and 60.1 μg/ml *in vitro,* respectively. The structural determination of **1** was accomplished by spectroscopic methods as well as X-ray diffraction analysis.

2. Results and discussion

Compound **1** was obtained as light yellow prisms (EtOAc) with mp 203–204°C and $[\alpha]_D^{20}$ +120.5 (*c* 0.044, $CHCl_3$). The HR-EI-MS of **1** established its molecular formula as $C_{20}H_{26}O_4$, indicating eight degrees of unsaturation. ^{13}C NMR and DEPT spectra showed 20 carbon signals including four methyls, five methenes, two methenyls, and nine quaternary carbons. The IR spectrum showed absorption bands of hydroxyl (3405 cm^{-1}, br), phenyl (1615, 1588 and 1461 cm^{-1}), and aromatic conjugated carbonyl (1640 cm^{-1}) groups, which were also confirmed by the ^{13}C NMR spectrum [δ_C 110.7 (s), 139.9 (d), 131.2 (d), 155.8 (s), 115.5 (s), 156.2 (s), and 204.8 (s)] and UV data [351.6 nm (4.67), 295.8 nm

*Corresponding author. Email: zhuchenchen@vip.sina.com

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(4.93), and 241.8 nm (4.75)]. Meanwhile the ^{13}C NMR spectral data [δ_C 81.0 (t), 155.8 (s)] indicated the presence of an epoxy functional group. Consideration of the predominant abietane diterpenoids isolated from *I. lophanthoides*, together with the typical ^1H NMR signals of four distinct methyl groups at δ_H 1.38 (d, 3H, 6.0), 0.94 (s, 3H), 0.96 (s, 3H), 1.35 (s, 3H), and conjugated carbonyl group [δ_C 204.8 (s)], and an intramolecular hydrogen bond [δ_H 13.4 (s, 1H)] indicated that **1** has an abietane diterpenoid skeleton. All of the H and C signals were assigned by HMQC spectrum (Table 1). The correlations of $^1H-^1H$ COSY suggested three segments in Figure 2. Key HMBC correlations from H_3-20 to C-10 and C-9, H_3-19 to C-3, C-4, and C-5, H_3-18 to C-3, C-4, and C-5 revealed 18, 19, and 20 methyls connected to the C-10, C-4, and C-4. The correlations of H-15 to C-13, C-14, and C-12, and H-16 to C-12 and C-13 indicated the presence of an epoxy between C-12 and C-16. According to the deduction mentioned above, the planar structure of **1** was determined (Figure 1). Single-crystal X-ray diffraction analysis was used to confirm the structure and its relative configuration (Figure 2). The results are shown in Figure 3 and in the experimental section. The X-ray structure showed that H-15 and CH_3-19, CH_3-20 are on the same orientation, while CH_3-17 and H-5 and CH_3-18 are on the opposite face. Thus, the relative configuration of three chiral carbons were determined as 5*S**, 10*S**, and 15*R**.

Compounds **1** and **2** have been tested for their inhibitory activities on NO production in LPS and IFN-γ activated murine macrophage-like cell line RAW 264.7. Gerardianin A (**1**) and 6,7- dehydroroyleanone (**2**) showed weak NO production inhibitory activity with IC_{50} values of 20.5 and 60.1 μg/ml, respectively, *in vitro.*

3. Experimental

3.1 General experimental procedures

Melting points were determined using an X6 micromelting points apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 341 polarimeter and UV spectra were obtained on a Shimadzu UV-240 spectrophotometer. IR spectra were recorded with an EQUINOX55 (Bruker, Billerica, MA) spectrophotometer. NMR spectra were recorded with a Varian Unity INOVA spectrometer 500 MHz and Bruker Unity BRUKER 400 MHz. HRMS spectra were obtained with a Thermo MAT 95XP mass spectrometer. The X-ray diffraction data were collected on a Bruker SMART 1000 CCD X-ray diffractometer.

3.2 Plant material

The aerial parts of *I. lophanthoides* var. *gerardianus* [Bentham] H. Hara (*Labiatae*) were collected from the GAP Planting Bases of Xihuangcao, Qingyuan City, Guangdong Province, China, in 2005. A voucher specimen (05-ILG) is deposited in the School of Chinese Medicine, Guangzhou University of Traditional Chinese Medicine.

3.3 Inhibitory activity on NO production from activated macrophage-like cell line, RAW 264.7

The cells were seeded at 1.2×10^6 cells/ml onto 96-well flat bottom plate (Sumitomo Bakelite, # 8096r, Tokyo) and then incubated at 37°C for 2 h. Next, the test extract was added to the culture simultaneously with both *Escherichia coil* LPS (100 ng/ml) and recombinant mouse IFN-γ (0.33 ng/ml). Then cells were incubated at 37°C for approximately 16 h and subsequently chilled on ice. One hundred micro liters of culture supernatant was placed in duplicate in the wells on the same plate. To quantify nitrite, 50 μl of Griess reagent (1% sulfanilamide in 5% H_3PO_4 and 0.1% *N*-1-naphthylethylene-iamide dihydrocholoride) was added to each well. After 10 min the reaction products were colorimetrically quantified at 550 nm using a Model 3550 Microplate Reader (BIO-RAD, Hercules, CA, USA) and the background absorbance (630 nm) was subtracted. Cytotoxicity was

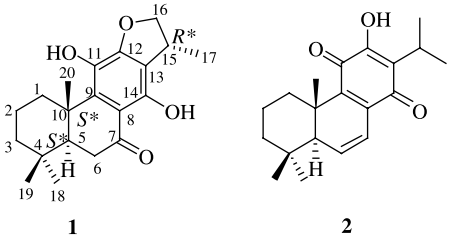
Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data ($CDCl_3$) for **1**.

Positions	δ_H (J in Hz)	δ_C mult	Positions	δ_H (J in Hz)	δ_C mult
1	3.26 br d (10.4) 1.50 m	36.5 (CH_2)	11		131.2 (C)
2	1.74 m, 1.60 m	19.1 (CH_2)	12		155.8 (C)
3	1.55 m, 1.24 m	41.2 (CH_2)	13		115.5 (C)
4		33.4 (C)	14		156.2 (C)
5	1.80 m	49.9 (CH)	15	3.72 m	35.5 (CH)
6	2.60 m, 2.58 m	35.6 (CH_2)	16	4.76 t (8.8) 4.26 dd (8.8, 6.0)	81.0 (CH_2)
7		204.8 (C)	17	1.38 d (6.0)	18.6 (CH_3)
8		110.7 (C)	18	0.94 s	33.2 (CH_3)
9		139.9 (C)	19	0.96 s	21.6 (CH_3)
10		40.7 (C)	20	1.35 s	18.2 (CH_3)

measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay method.

3.4 Extraction and isolation

The aerial parts of *I. lophanthoides* var. *gerardianus* (5.0 kg, dried wt) were extracted with hot EtOH (251 × 3). The combined extracts were concentrated under reduced pressure to afford a brown extract (500 g). The EtOH extract was partitioned between EtOAc and H_2O. The EtOAc soluble portion (152 g) was chromatographed on silica gel column using petroleum ether (PE)–$CHCl_3$ mixtures of increasing polarity as eluting solvent to yield 12 fractions. The second fraction was eluted with PE/$CHCl_3$, 9:1. It was further separated by using flash chromatography on silica gel (eluted with PE/$CHCl_3$, 95:5) to afford 6,7-dehydroroyleanone (**2**) (68.8 mg). The third fraction (eluted with PE/$CHCl_3$, 5:1) was purified by using flash chromatography on silica gel eluted with EtOAc/PE (10:90) to afford gerardianin A (**1**) (60.1 mg).



1 **2**

Figure 1. Structures of compounds **1** and **2**.

3.4.1 Gerardianin A (*1*)

Light yellow prisms (EtOAc); mp 203–205°C; $[\alpha]_D^{20}$ +120.5 (*c* 0.044, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 351.6 (4.67), 295.8 (4.93), 241.8 (4.75) nm; IR (KBr) ν_{max} 3247 (—OH), 2919, 2850, 1640 (s, C═O), 1615, 1588, 1461, 1403, 1355, 1310, 1255, and 947 cm^{-1}; FAB-MS *m/z* 331 $[M + H]^+$; HR-EI-MS *m/z* 330.1828 $[M]^+$ (calcd for $C_{20}H_{26}O_4$, 330.1826); ^{1}H NMR and ^{13}C NMR spectral data, see Table 1.

3.4.2 X-ray crystal structure analysis of gerardianin A (*1*)

A prismatic crystal of gerardianin A (**1**) was selected for data collection on a Bruker Smart 1000 CCD diffractometer at $T = 173$ (2) K with graphite monochromatized Mo Kα radiation ($\lambda = 0.71073$ Å) and processed with SAINT-Plus. Absorption corrections were calculated by using SADABS. The structure was solved by direct methods and refined by full-matrix least-squares methods on F^2 using SHELXTL. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included at

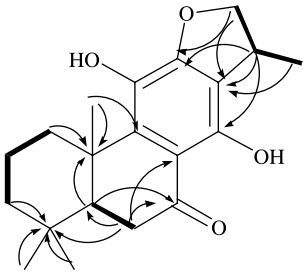


Figure 2. Key $^1H - {}^1H$ COSY (bold lines) and HMBC correlations (H → C) of **1**.

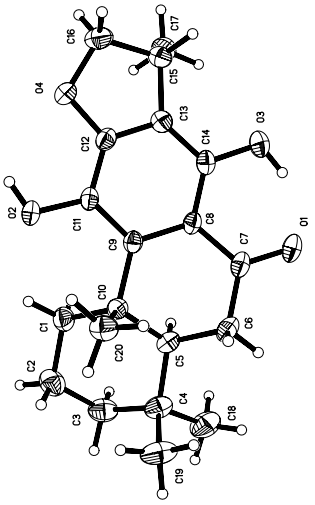


Figure 3. X-ray crystal structure of **1**.

idealized positions and refined by using the riding model. Crystal data for $C_{20}H_{26}O_4$: crystal dimensions $0.48 \times 0.43 \times 0.29\,mm^3$, orthorhombic space group $P2_1$, $a = 8.3515(5)$ Å, $b = 9.1052(5)$ Å, $c = 22.7573(13)$ Å, $\alpha = 90°$, $\beta = 90°$, $\gamma = 90°$, $V = 1730.51(17)$ Å^3, $Z = 4$, $\rho_{calcul} = 1.268\,mg/m^3$. Of the 8098 reflections measured ($2.41 \ \theta \leq 27.07°$), 2175 independent reflections were used to solve and refine the structure. Based on all the data and 223 refined parameters, final $R_1 = 0.0439$, ${}_wR_2 = 0.0967$, and the god-of-fit on $F^2 = 1.061$. A final difference Fourier map showed no significant residual electron density, the largest difference peak and hole being 0.282 and 0.152 e/Å^3, respectively.

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