Two New Drimane Sesquiterpenoids from Compound Changweikang and Their Inhibitory Activity against Nitric Oxide Production

Ming-sheng LIU,*[a,b] Chang LIU,[c] Xiao-po ZHANG,[a] Lin SHENG,[a,b] Jun-qing ZHANG,[a,b] and Sheng-li KANG[a,b]

[a] *School of Pharmaceutical Sciences, Hainan Medical University; Haikou 571101, P.R. China:* [b] *Hainan Provincial Key Laboratory of R&D of Tropical Herbs; Haikou 571101, P.R. China: and* [c] *School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University; Shenyang 110016, P.R. China.*

Received March 3, 2010; accepted May 8, 2010; published online June 18, 2010

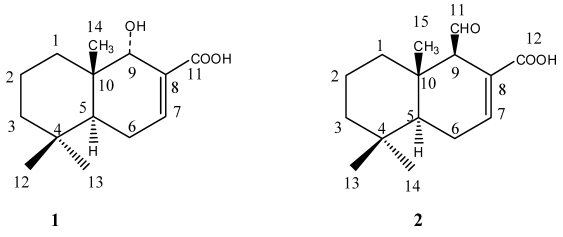
Two new drimane sesquiterpenoids, changweikangic acid A (1) and B (2), were isolated from Compound Changweikang. Their structures were established on basis of extensive spectroscopic analyses including two dimensional (2D) NMR and X-ray crystallographic data. The two compounds were evaluated for their inhibitory activity against nitric oxide production in lipopolysaccharide-activated macrophage cell line, RAW 264.7 cells.

Key words compound Changweikang; *Daphniphyllum calycinum*; *Polygonum hydropiper*; changweikangic acid A; changweikangic acid B; nitric oxide production

Compound Changweikang (CC) is a sort of Chinese patent drug, which is made from the aqueous extract of two medicinal plants of *Daphniphyllum calycinum* BENTH. and *Polygonum hydropiper* L. CC possessing the effect of heat-clearing and has been used clinically for treatment of various gastroenteritis and its preparative processes are as follows: the two plants were combined (2 : 1) and extracted by decoction with water, then the aqueous extract was concentrated to dryness. Finally, CC was prepared by adding pharmaceutic adjuvant to the obtained extract.[1)] Our previous studies demonstrated that this prescription showed anti-inflammatory and spasmolytic properties.[2)] As a part of our ongoing study to search for active compounds from this prescription, two new drimane sesquiterpenoids (**1**, **2**) were isolated. This paper deals with the isolation and structural elucidation of the two compounds, as well as their inhibitory activities against nitric oxide production in activated macrophages.

Results and Discussion

Compound **1** was obtained as colorless needles. Its molecular formula was established as $C_{14}H_{22}O_3$ by positive high-resolution (HR)-electrospray-ionization (ESI)-time-of-flight (TOF)-MS (*m*/*z* 261.1466 $[M+Na]^+$, Calcd for 261.1467). The IR spectrum showed main absorptions at 3455.8 (OH) and 1702.8 cm^{-1} (conjugated carbonyl group). ^1H- and ^{13}C-



1 **2**

Fig. 1. Structures of **1** and **2**

Table. 1. ^1H- and ^{13}C-NMR Chemical Shifts of **1** and **2** in DMSO-d_6[a)]

No.	**1**		**2**[b)]	
	H	C	H	C
1	α 1.75 (1H, td, 13.2, 3.3) β 1.02 (1H, br d, 13.2)	33.6	α 1.29 (1H, dt, 13.2, 2.4) β 1.65 (1H, d, 13.2)	37.2
2	α 1.41 (1H, br d, 12.0) β 1.54 (1H, td, 12.0, 3.6)	18.1	α 1.38 (1H, m) β 1.57 (1H, td, 12.0, 3.6)	18.1
3	α 1.13 (1H, td, 13.8, 4.2) β 1.36 (1H, d, 13.8)	42.2	α 1.10 (1H, td, 13.8, 4.2) β 1.40 (1H, m)	41.8
4		32.3		32.6
5	1.57 (1H, m)	40.1	1.42 (1H, m)	43.1
6	α 2.20 (1H, dt, 19.8, 4.5) β 1.96 (1H, m)	22.9	α 2.32 (1H, dt, 19.8, 4.8) β 2.07 (1H, m)	24.4
7	6.85 (1H, dd, 4.5, 2.4)	140.8	7.13 (1H, dd, 3.6, 1.2)	142.3
8		132.2		126.3
9	3.54 (1H, s)	71.4	3.06 (1H, d, 1.5)	60.6
10		36.9		36.7
11		168.0	9.74 (1H, d, 1.5)	203.7
12	0.87 (3H, s)	21.4		167.5
13	0.85 (3H, s)	32.6	0.87 (3H, s)	21.4
14	0.65 (3H, s)	18.4	0.85 (3H, s)	32.5
15			1.10 (3H, s)	21.1
–COOH	12.01 (1H, br s)			

a) Coupling constants (*J* in Hz) are given in parentheses. *b*) ^1H- and ^{13}C-NMR were measured in 600 MHz and 150 MHz separately.

* To whom correspondence should be addressed. e-mail: mingsliu2002@yahoo.com

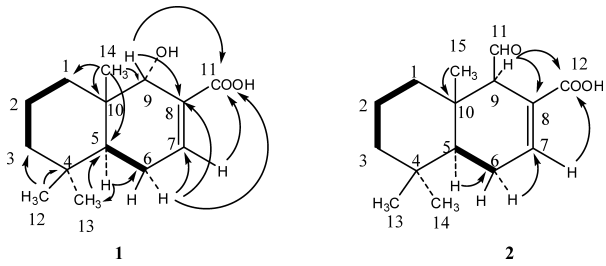


Fig. 2. ^{1}H–^{1}H COSY (Bold Lines) and HMBC (Full-Line Arrows) Correlations of **1** and **2**

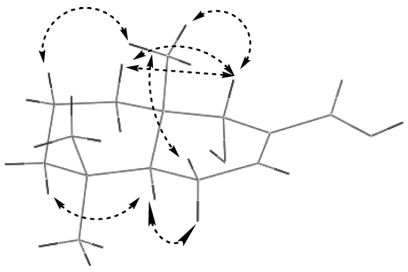


Fig. 3. Important NOE Correlations Observed in **1**

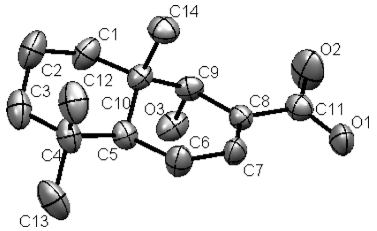


Fig. 4. The X-Ray Crystallographic Structure of **1**

NMR spectra of **1** (Table 1) exhibited signals characteristic for the presence of three methyl groups [δ_H 0.87, 0.85, 0.65 (3H, each, s)], one carboxyl group [δ_H 12.01 (1H, br s), δ_C 168.0], one olefinic proton [δ_H 6.85 (1H, dd, J=4.5, 3.0 Hz, H-7)], and one oxymethine group [δ_H 3.54 (1H, s, H-9), δ_C 71.4]. The UV absorption (λ_{max} 220 nm) along with the ^{13}C-NMR spectral data at δ 140.8 (C-7), 132.2 (C-8), and 168.0 (C-11) implied the presence of one α,β-unsaturated carboxylic group.[3] The gross structure of **1** was established by analyses of its two dimensional (2D) NMR experiments including ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple-bond correlation (HMBC). The ^{1}H–^{1}H COSY and HSQC spectra of **1** suggested the existence of two units as shown in Fig. 2. In the HMBC experiments, correlations for H_2-6 (δ 1.96, 2.20) and H-9 (δ 3.54) to C-7 (δ 140.8), C-8 (δ 132.2), C-11 (δ 168.0) revealed that C-6 (δ 22.9) and C-9 (δ 71.4) were attached to C-7 and C-8 of the α,β-unsaturated carboxylic group, separately. HMBC cross-peaks between H_3-14 (δ 0.65) and C-10 (δ 36.9)/C-1 (δ 33.6)/C-5 (40.1)/C-9 (δ 71.4) indicated that C-14, C-1, C-9, and C-5 were all connected to C-10. Cross-peaks between H_3-12 (δ 0.87)/H_3-13 (δ 0.85) and C-3 (42.2)/C-4 (32.3)/C-5 (40.1) suggested that C-12, C-13, C-3, and C-5 were all attached to C-4. Thus, the planar structure of **1** was established. The stereochemistry of **1** was determined by nuclear Overhauser effect spectroscopy (NOESY) experiments. NOE correlations observed between H_β-1 and H_3-14, H_β-2 and H_3-14, and between H-5 and H_α-3/H_α-6 implied a *trans*-junction for the two rings (Fig. 3).[4] Correlations between H-9 and H_β-1/H_3-14 indicated that the hydroxy group at C-9 was located at α configuration. Finally, combined with single X-ray diffraction (Fig. 4), the structure of **1** was determined to be changweikangic acid A as shown in Fig. 1.

Compound **2** was obtained as colorless needles. Its molecular formula was determined to be $C_{15}H_{22}O_3$ by HR-ESI-TOF-MS. The IR spectrum of **2** showed the presence of a chelate hydroxy group (3452.4 cm^{-1}), an aldehyde group (1719.7 cm^{-1}), and a conjugated carbonyl group (1676.8 cm^{-1}). The UV spectrum together with the ^{1}H- and ^{13}C-NMR spectral data indicated that compound **2** was also a drimane sesquiterpenoid possessing the same skeleton as **1**. Detailed comparison of its NMR data with those of polygodial showed that they shared many spectral features in common.[5] However, the aldehyde group at C-8 observed in polygodial is replaced by one carboxyl group [δ_H 12.00 (1H, br s, H-12), δ_C (167.5)] in **2**. This change was confirmed by the positive bromcresol green reaction and HMBC correlations between H-7 and C-12, and between H-9 and C-12. In the NOESY experiments, cross peaks between H_β-1 and H_3-15, H_β-2 and H_3-15, and between H-5 and H_α-3/H_α-6 implied that the rings junction was *trans*. Correlations noted between H-9 and H_α-1/H-5 indicated that the aldehyde group was β-oriented. Based on these and the similar optical rotations between **2** and polygodial, a conclusion can be drawn that the two compounds shared the same sterostructure. Finally, **2** was determined to be changweikangic acid B as shown in Fig. 1.

Table 2. Inhibitory Effect of **1** and **2** on NO Production

Groups	Dose (μM)	Inhibition rate (%)
1	0.001	74.48
	0.01	72.54
	0.1	87.55
2	0.001	76.98
	0.01	87.76
	0.1	100.0
Aminoguanidine[a)]	0.025	50

a) Positive control.

HPLC analysis showed that both **1** and **2** were found in CC and the aqueous extract of *P. hydropiper*, but not found in aqueous extract of *D. calycinum*.

Nitric oxide (NO) is a small molecule compound with widespread and complicated biological activity, taking part in the regulation of many physiological functions, such as host defense and neurotoxicity. However, the excess production of NO has been implicated for immunological and inflammatory diseases including septic shock, rheumatoid arthritis, and graft rejection. Therefore, inhibition of NO production is apparently an important way in curing many diseases.[6] Compounds **1** and **2** were evaluated for their inhibitory activities against nitric oxide production and they showed notable activity which was stronger than that of aminoguanidine used as a positive control, see Table 2.

Experimental

Procedures Optical rotations were obtained using a HHW5/ATR-W2 digital polarimeter (SCHMIDT+HAENSCH, German). Melting points were determined on an XT-5 melting point apparatus (Shanghai, P. R. China). IR

spectra were obtained on a JASCO-FT-IR-4100 infrared spectrophotometer (Japan). UV spectra were taken on a JASCO-UV-V650 spectrophotometer (Japan). HR-ESI-TOF-MS data were recorded on a Waters LCT Premier XE spectrometry. The NMR data were recorded on a Bruker AV-600 spectrometer (600 MHz for ^{1}H and 150 MHz for ^{13}C) in dimethylsulfoxide (DMSO)-d_6 with tetramethylsilane (TMS) as an internal standard. Column chromatography was performed on silica gel (200—300 mesh, Qingdao Haiyang Chemical Co., Ltd., Qingdao, P. R. China) and Sephadex LH-20 (Pharmacia Biotech Sweden).

Plant Material The two medicinal plants of *D. calycinum* and *P. hydropiper* were collected from Hainan Island, China in April 2007. Voucher specimens (DC200704 and PH200704) were identified by Prof. Wei-ping Chen and deposited in School of Pharmaceutical of Science, Hainan Medical University.

Extraction and Isolation The air-dried *D. calycinum* (14 kg) and *P. hydropiper* (7 kg) were combined and extracted with water after grounding into powder. The solvent was removed under reduced pressure to afford an extract (170 g). Then the extract was successively partitioned with dichloromethane. The dichloromethane extract (54 g) was subjected to silica gel chromatography, eluting with a gradient of petroleum ether-acetone (1 : 0—0 : 1, v/v), to give eight fractions (Frs. 1—8). Fr. 3 (600 mg) was applied to silica gel H column chromatography, eluting with hexane–ethyl acetate (4 : 1) to afford **2** (250 mg). Fr. 4 was applied to silica gel H column chromatography, eluting with a gradient of hexane–acetone (10 : 1—3 : 1, v/v) to give five fractions (Frs. A—E), Fr. D (500 mg) was separated by a column of Sephadex LH-20 to afford **1** (150 mg).

Changweikangic Acid A (**1**): Colorless needles; mp 188—190 °C; $[\alpha]_D^{20}$ +14 (c=0.2, MeOH), IR νmax (KBr) cm^{-1}: 3455.8, 2928.0, 1702.8, 1652.7, 1419.4, 1032.7; UV λ_{max} 220 (MeOH) nm (ε): 220 (3.83); ^{1}H-NMR (DMSO-d_6, 600 MHz) and ^{13}C-NMR (DMSO-d_6, 150 MHz): see Table 1. HR-ESI-TOF-MS: m/z 261.1466 $[M+Na]^+$ (Calcd for $C_{14}H_{22}O_3Na$, 261.1467).

HPLC Analysis Conditions The two compounds and the aqueous extracts of CC and the two medicinal plants were analyzed on a Phenomenex Luna C_{18} column (250×4.6 mm, 5 μm) using methanol (A)–0.2% phosphoric acid solution (B) in gradient mode (the initial ratio of solution A was 70% and decreased to 53% within 12 min, and the ratio of solution A was maintained 53% within following 33 min) as the mobile phase, the flow rate was 1.0 ml·min^{-1}, the column was maintained at 30 °C and the detective wavelength was set at 220 nm. The retention time of **1** and **2** were 36.762 and 41.024 min, separately.

Single-Crystal X-Ray Structure Determination Crystal **1** was crystallized from MeOH. Crystal data: $C_{14}H_{22}O_3$, MW 238.32, triclinic, space group P-1, a=7.4784(7) Å, b=7.4915(7) Å, c=12.8876(13) Å, α=100.421°, β=90.817°, γ=107.985°, V=642.6(3) Å^3, Z=2, D_{calc}=1.175 g/cm^{-3}, F(000)=260, T=298 K, crystal size 0.48×0.41×0.17 mm. A suitable crystal of **1** was selected and mounted on a Bruker Smart-1000 CCD diffractometer for data collection which was performed on a graphite-monochromatized Mo$K\alpha$ (λ=0.71073 Å) radiation using an ω scan mode. Data collection yielded 3512 reflections, 2333 were independent. The structure was solved by direct methods with the use of the SHELX-97 program, and the non-hydrogen atoms were refined anisotropically by full-matrix least-squares calculations on F^2. Final R-factors ($I>2\sigma(I)$) were R=0.0492 and $wR2$=0.0973.

Changweikangic Acid B (**2**): Colorless needles; mp 120—122 °C; $[\alpha]_D^{20}$ −24 (c=0.1, $CHCl_3$); IR ν_{max} (KBr) cm^{-1}: 3452.4, 2927.5, 1719.7, 1676.8, 1647.3, 1421.4, 1265.7, 1020; UV λ_{max} 220 (MeOH) nm (ε): 224 (4.10); ^{1}H-NMR (DMSO-d_6, 600 MHz) and ^{13}C-NMR (DMSO-d_6, 150 MHz): see Table 1. HR-ESI-TOF-MS: m/z $[M-H]^-$ 249.1485 (Calcd for $C_{15}H_{21}O_3$, 249.1491).

NO Production from Lipopolysaccharide (LPS)-Stimulated Macrophages Inhibitory effect on the NO production by mouse macrophages (RAW 264.7) was evaluated using the method reported previously.[7] Briefly, thioglycolate (TGC)-induced peritoneal exudate cells (5105 cells/well) were collected from the peritoneal cavities of male ddY mice and were suspended in 100 ml of RPMI 1640 supplemented with 5% fetal calf serum (FCS), penicillin G (100 units/ml) and streptomycin sulfate (100 mg/ml), and precultured in 96 well microplates at 37 °C in 5% CO_2 in air for 1 h. Nonadherent cells were removed by washing with phosphate buffer solution (PBS), and the adherent cells were cultured in 200 ml of fresh medium containing 10 mg/ml LPS and various concentrations of each test compound for 20 h. NO production in each well was assessed by measuring the accumulation of nitrite (NO_2^-) in the culture medium using Griess reagent.

$$\text{inhibition (\%)} = [(A-B)/(A-C)] \times 100\%$$

A−C: NO_2^- concentration (μM)
[A: LPS (+), sample (−); B: LPS (+), sample (+); C: LPS (−), sample (−)].

Acknowledgements This study was supported by National Key Technology R&D Program during the 11th Five-Year Plan Period (No. 2007BAI2704).

References

1) Liu C., Yuan Z., Liu M. S., Fu N. G., *Chin. Trad. Pat. Med.*, **31**, 1578—1581 (2009).
2) Tan Y. F., Li H. L., Zhang J. Q., Liu M. S., *Lishizhen. Med. Mat. Med. Res.*, **20**, 2941—2942 (2009).
3) Sakio Y., Hirano Y. J., Hayashi M., Komiyama K., Ishibashi M., *J. Nat. Prod.*, **64**, 726—731 (2001).
4) Iijima T., Yaoita Y., Kikuchi M., *Chem. Pharm. Bull.*, **51**, 545—549 (2003).
5) De Almeida Alves T. M., Ribeiro F. L., Kloos H., Zani C. L., *Mem. Inst. Oswaldo. Cruz.*, **96**, 831—833 (2001).
6) Su J., Wu Z. J., Zhang W. D., Zhang C., Li H. L., Liu R. H., Shen Y. H., *Chem. Pharm. Bull.*, **56**, 589—591 (2008).
7) Matsuda H., Morikawa T., Ando S., Oominami H., Murakami T., Kimura I., Yoshikawa M., *Bioorg. Med. Chem.*, **12**, 3037—3046 (2004).