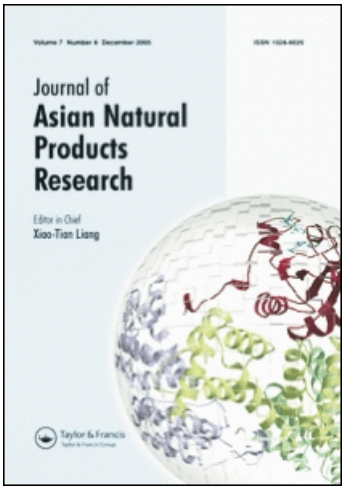

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Fu-Lin Yan[a]; Lan-Qing Guo[a]; Chun-Ming Wang[b]; Ji-Xia Zhang[a]
[a] Pharmacy College, Xinxiang Medical University, Xinxiang, China [b] School of Life Sciences, Lanzhou University, Lanzhou, China

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Chemical constituents of *Isodon nervosus* and their cytotoxicity

Fu-Lin Yan[a]*, Lan-Qing Guo[a], Chun-Ming Wang[b] and Ji-Xia Zhang[a]

[a]*Pharmacy College, Xinxiang Medical University, Xinxiang 453003, China;* [b]*School of Life Sciences, Lanzhou University, Lanzhou 730000, China*

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Two new *ent*-kaurane diterpenoids, 6,20,15α-trihydroxy-6,7-*seco*-1α,7-olide-*ent*-kaur-16-ene (**1**) and 7β,12α-dihydroxy-6β,15β-diacetoxy-7α,20-epoxy-*ent*-kaur-2,16-dien-1-one (**2**), together with the six known compounds, were isolated from the aerial part of *Isodon nervosus.* The structures of the new compounds were determined by spectral methods (1D, 2D NMR, and MS). Six compounds were assayed for their cytotoxicity against HL60, SMMC-7721, and HeLa human cell lines. Compounds **5**, **7**, and **8** showed significant cytotoxicity.

Keywords: *Isodon nervosus*; *ent*-kaurane diterpenoid; cytotoxicity; Labiatea

1. Introduction

Isodon nervosus (Labiatae) is widely distributed in China, and has long been used as a Chinese folk medicine in the treatment of acute jaundice, hepatitis, and acute cholecystitis [1]. Previous study of this plant yielded some *ent*-kaurane diterpenoids, most of them have been shown to possess antitumor and anti-inflammatory activities [2,3]. We re-examined the leaves of *I. nervosus* collected in Henan Province of China and obtained two new *ent*-kaurane diterpenoids (**1** and **2**) and six known compounds (**4**–**9**) (Figure 1). In addition, six compounds were tested for their cytotoxicity toward human leukemia cell (HL60), human hepatoma cell (SMMC-7721), and human cervical carcinoma cell (HeLa). This report describes the structure determination of compounds **1** and **2**.

2. Results and discussion

The EtOAc soluble part of Me_2CO/H_2O (7:3, v/v) extract from the leaves of *I. nervosus* was chromatographed repeatedly over silica gel column to afford two new *ent*-kaurane diterpenoids and six known compounds. The known compounds were identified as epinodosinol (**4**) [4], isodocarpin (**5**) [5], epinodosin (**6**) [4], effusanin A (**7**) [6], effusanin E (**8**) [6], and 1-*O*-β-D-glucopyranosyl-(2*S*,3*S*,4*R*,5*E*,9*Z*)-2-*N*-(2′-hydroxyl-tetracosanoyl)1,3,4-trihydroxy-5,9-octadienine (**9**) [7] by the comparison of their spectral data (^{1}H, ^{13}C NMR, DEPT, and MS) with those reported in the literature, respectively.

Compound **1** was obtained as colorless needles, and the molecular formula of $C_{20}H_{30}O_5$ was determined on the basis of HR-ESI-MS at *m/z* 373.1992 $[M+Na]^+$. In its IR spectrum, the absorption bands at 3420, 3347, 3253, and 1695 cm^{-1} showed the presence of hydroxyl groups and an ester carbonyl group. In addition, the ^{13}C NMR and DEPT spectra exhibited the signals of two tertiary methyls, eight methylenes (including two oxygenated at δ 59.6 (t), 61.1 (t) and one olefinic carbon at δ 108.5 (t)), five methines

*Corresponding author. Email: yanfulin03@xxmu.edu.cn

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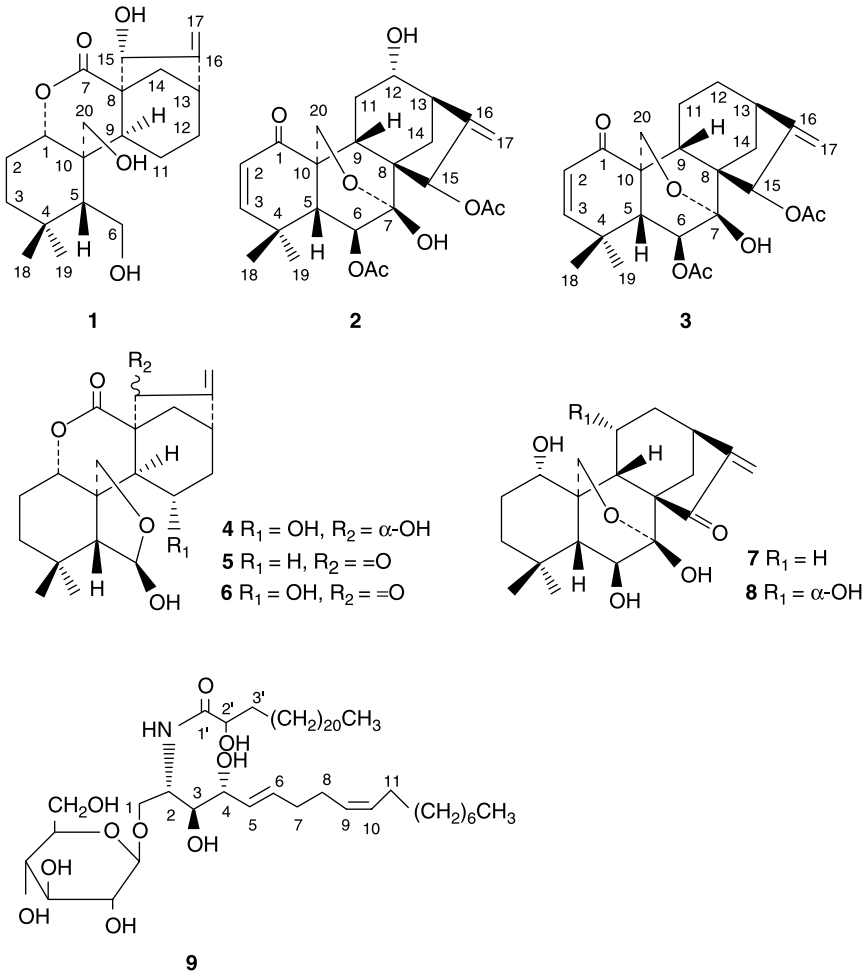

Figure 1. The structures of compounds **1**–**9**.

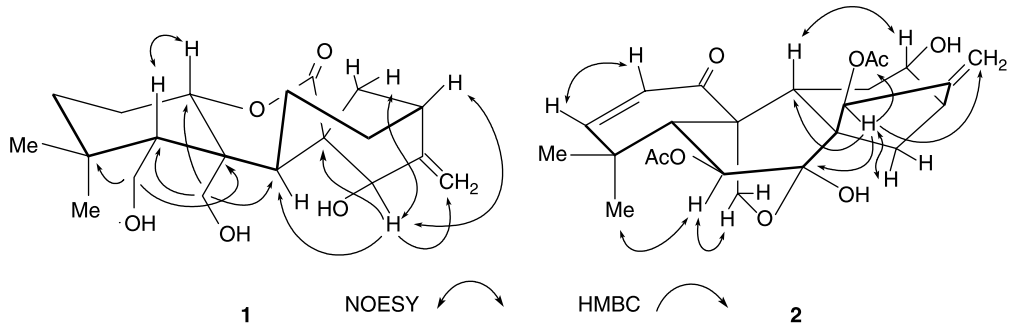

Figure 2. The key NOESY and HMBC correlations of compounds **1** and **2**.

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data of compounds **1** and **2**[a].

Position	**1**, δ_C	**1**, δ_H	**2**, δ_C	**2**, δ_H
1	78.6 d	4.51 (1H, m)	197.3 s	
2	25.3 t	1.41, 1.85 (each 1H, m)	128.0 d	6.06 (1H, d, $J = 10.0$ Hz)
3	39.9 t	1.36 (2H, m)	159.8 d	6.69 (1H, d, $J = 10.0$ Hz)
4	34.3 s		35.7 s	
5	50.6 d	1.68 (1H, m)	51.7 d	2.79 (1H, d, $J = 9.2$ Hz)
6	59.6 t	4.04, 4.21 (ABd, each 1H, $J = 11.6$ Hz)	73.5 d	5.90 (1H, d, $J = 9.2$ Hz)
7	176.0 s		96.1 s	
8	52.0 s		52.5 s	
9	34.3 d	3.13 (1H, dd, $J = 4.4$, 13.6 Hz)	39.0 d	2.38 (1H, dd, $J = 4.8$, 13.6 Hz)
10	45.3 s		46.5 s	
11	17.8 t	1.56, 1.94 (each 1H, m)	29.7 t	2.33, 2.53 (each 1H, m)
12	33.1 t	2.01, 2.11 (each 1H, m)	75.7 d	4.09 (1H, m)
13	37.1 d	2.66 (1H, m)	47.5 d	2.96 (1H, m)
14	33.7 t	1.71, 1.98 (each 1H, m)	25.4 t	2.24 (1H, dd, $J = 4.8$, 12.4 Hz), 2.66 (1H, d, $J = 12.4$ Hz)
15	79.5 d	5.58 (1H, s)	75.8 d	6.19 (1H, s)
16	159.9 s		154.1 s	
17	108.5 t	5.18, 5.47 (each 1H, brs)	111.0 t	5.21 (2H, brs)
18	34.2 q	1.26 (3H, s)	29.4 q	1.10 (3H, s)
19	23.0 q	1.18 (3H, s)	24.5 q	1.15 (3H, s)
20	61.1 t	4.10, 4.24 (each 1H, ABd, $J = 11.6$ Hz)	65.5 t	4.22, 4.67 (each 1H, d, $J = 9.6$ Hz)
OAc			21.1 q, 170.7 s	2.14 (3H, s)
			21.9 q, 170.9 s	2.28 (3H, s)

[a] The spectra of **1** and **2** were measured in C_5D_5N, TMS as the internal standard.

Table 2. Cytotoxic activities of compounds **4**–**9**.

Compound	IC_{50} (μg/ml)		
	HL60	SMMC-7721	HeLa
4	26.44 ± 1.99	39.68 ± 5.64	59.90 ± 1.69
5	0.57 ± 0.12	3.57 ± 0.26	4.01 ± 0.67
6	2.08 ± 0.51	17.64 ± 0.07	40.76 ± 5.15
7	0.26 ± 0.10	1.45 ± 0.25	7.67 ± 0.24
8	0.55 ± 0.25	23.69 ± 2.74	48.87 ± 4.50
9	>400	>400	>400
Mitomycin	0.56 ± 0.17	1.85 ± 0.53	1.11 ± 0.64

(including two oxygenated at δ 78.6 (d) and 79.5 (d)), and five quaternary carbon (including one olefinic carbon at δ 159.9 (s) and one δ-lactone carbon at δ 176.0 (s)). Comparison of the ^{13}C chemical shifts of **1** with those of compounds **4**, **5**, and **6**, the basic skeleton of **1** was considered to be a 6,7-*seco*-1,7-olide-*ent*-kaur-16-ene. Compound **1** differed from **4** mainly by a lack of OH at 11-position and 6-hemiacetal. In the ^{13}C NMR and DEPT spectra of **1**, two oxygenated methylenes at δ 59.6 (t) and 61.1 (t) were there instead of the signal for 6-hemiacetal (δ 102.3) in **4**. This was supported by the HMBC (Figure 2) correlations between H-6 (δ 4.04 and 4.21) and C-4 (δ 34.3), C-5 (δ 50.6), C-10 (δ 45.3); H-20 (δ 4.10 and 4.24) and C-1 (δ 78.6), C-5 (δ 50.6), C-9 (δ 34.3). The observed NOE (Figure 2) correlations between H-15 and H-14β (strong), between H-15 and H-13β (weak), and between H-9 and H-20, showed that the H-15 was in β-orientation and 20-CH_2OH was in α-orientation. Based on the above evidence, the structure of **1** was elucidated as 6,20,15α-trihydroxy-6,7-*seco*-1α,7-olide-*ent*-kaur-16-ene.

Compound **2** isolated as colorless needles, exhibited a molecular formula $C_{24}H_{30}O_8$ based on its HR-ESI-MS at *m/z* 469.1818 $[M+Na]^+$. The IR spectrum exhibited the presence of carbonyl groups (1725, 1716, and 1661 cm^{-1}) and hydroxyl groups (3535 and 3445 cm^{-1}). In the ^{13}C NMR and DEPT spectra (Table 1) of **2**, besides four carbon signals for two acetoxyl groups, there were 20 signals for the skeleton of a 7,20-epoxy-*ent*-kaurane deduced from the characteristic signals of two methyls (C-18 (29.4, q) and C-19 (24.5, q)), three methines (C-5 (51.7, d), C-9 (39.0, d), and C-13 (47.5, d)), three quaternary carbons (C-4 (35.7, s), C-8 (52.5, s), and C-10 (46.5, s)), an oxymethylene (C-20 (65.5, t)), and a hemiketal group (C-7 (96.1, s)). Comparison of the ^1H and ^{13}C NMR spectral data of **2** with those of **3** [8] (odonicin, a known 7,20-epoxy-*ent*-kaurane from *Isodon japonicus*) indicated that **2** was identical with **3** except for the difference at C-12, suggested **2** as 12α-hydroxy-odonicin, which was supported by the HMBC and NOESY spectral evidences (Figure 2). The 12-OH was determined by the HMBC correlations of H-12 (δ 4.09, 1H, m) with C-14 (δ 25.4) and C-16 (δ 154.1), and the α-orientation of the 12-OH was ensured by the NOE (Figure 2) correlations of H-12 with H-9β (strong). Therefore, **2** was elucidated as 7β,12α-dihydroxy-6β,15β-diacetoxy-7α,20-epoxy-*ent*-kaur-2,16-dien-1-one.

The cytotoxic activity (Table 2) of six compounds was tested, and the results showed that isodocarpin (**5**), effusanin A (**7**), and effusanin E (**8**) exhibited significant cytotoxic activity against HL60 cell with IC_{50} values of 0.57, 0.55, and 0.26 μg/ml, respectively, while **5** and **7** exhibited appreciable cytotoxic activity against SMMC-7721 cell (IC_{50} values 3.57 and 1.45 μg/ml, respectively) and HeLa cell (IC_{50} values 4.01 and 7.67 μg/ml, respectively).

3. Experimental

3.1 General experimental procedures

Melting points were measured on a Kofler melting point instrument and are uncorrected. Optical rotations were taken on a Perkin-Elmer 341 polarimeter. The IR spectra were obtained on a Nicolet 170 SX FT-IR spectrometer. UV spectra were recorded on a Shimadzu UV-2550 instrument. ^{1}H, ^{13}C, and 2D NMR spectra were measured on a Bruker AM-400 NMR spectrometer using TMS as the internal standard. FAB-MS data were obtained on a VG-ZAB-HS mass spectrometer (at 70 eV); HR-ESI-MS were recorded on a Waters HPLCQ-Tof HR-MS spectrometer. Silica gel (200–300 mesh) used for column chromatography and silica gel GF_{254} for TLC was made by the Qingdao Marine Chemical Factory of China (Qingdao, China). Mitomycin used as a positive control was supplied by Zhejiang Hisun Pharmaceutical Co. Ltd (Taizhou, Zhejiang, China). Spots were detected on TLC under UV or by heating after spraying with 5% H_2SO_4 in C_2H_5OH.

3.2 Plant material

The leaves of *I. nervosus* were collected in Xinyang of Henan Province, China, in August 2007. It was identified by Prof. Changshan Zhu, Henan Agriculture University, China. A voucher specimen (No. 200708) is deposited in Pharmacy College, Xinxiang Medical University.

3.3 Extraction and isolation

The air-dried leaves of *I. nervosus* (12 kg) were pulverized and extracted with Me_2CO/H_2O (7:3, v/v), (four times, 5 days each time) at room temperature and filtered. The combined Me_2CO/H_2O (7:3, v/v) extract was concentrated under pressure and extracted with EtOAc, and then concentrated to obtain residue (270 g), which was absorbed on 500 g silica gel and subjected to silica gel column (10 × 100 cm, 200–300 mesh, 3000 g) gradiently eluted with $CHCl_3$/MeOH (1:0, 30:1, 20:1, 10:1, 5:1, 0:1) to give six fractions (1–6) according to their TLC analysis. Fraction 2 ($CHCl_3/CH_3OH$ 30:1) was chromatographed on silica gel column gradiently eluted with $CHCl_3/Me_2CO$ (20:1, 15:1) to afford compound **5** (43 mg). Fraction 3 ($CHCl_3/CH_3OH$ 20:1) was further separated on a silica gel column into three fractions (3a–c) by eluting with $CHCl_3$/MeOH (30:1, 20:1, 10:1). Compound **6** (38 mg) was obtained from fraction 3a by column chromatography with $CHCl_3/(CH_3)_2CHOH$ (30:1) as eluent and recrystallization in MeOH. Fraction 3b was chromatographed repeatedly over silica gel column and purified by recrystallizing in $CHCl_3–Me_2CO$ (20:1) to afford compounds **2** (7 mg) and **7** (75 mg). Compound **1** (8 mg) was obtained from fraction 3c by column chromatography with $CHCl_3/(CH_3)_2CHOH$ (30:1) and recrystallization in $CHCl_3/CH_3OH$ (15:1). From fraction 4 ($CHCl_3$—CH_3OH 10:1), compounds **4** (29 mg) and **8** (56 mg) were obtained by repeated silica gel column chromatography with $CHCl_3$/MeOH (20:1) and $CHCl_3/(CH_3)_2CHOH$ (20:1) successively. From fraction 5 ($CHCl_3–CH_3OH$ 5:1), compound **9** (1.1 g) was isolated by repeated silica gel column chromatography with $CHCl_3–CH_3OH$ (8:1) as eluent.

3.3.1 *6,20,15α-Trihydroxy-6,7-*seco-*1α,7-olide-*ent-*kaur-16-ene* (**1**)

Colorless needles; $C_{20}H_{30}O_5$; mp 218–220°C; α_D^{20} − 51 ($c = 0.14$, CH_3OH); IR (KBr) ν_{max} (cm^{-1}): 3420, 3347, 3253, 3015, 1695, 1456, 1433, 1372, 1338, 1233, 1137, 1118, 1098; ^{1}H and ^{13}C NMR spectral data are shown in Table 1; HR-ESI-MS *m/z*: 373.1992 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_5Na$, 373.1991).

3.3.2 *7β,12α-Dihydroxy-6β,15β-diacetoxy-7α,20-epoxy-*ent-*kaur-2,16-dien-1-one* (**2**)

Colorless needles; $C_{24}H_{30}O_8$; mp 209–211°C; α_D^{20} − 160 ($c = 0.16$, CH_3OH); UV (MeOH) λ_{max} ($\log \varepsilon$): 228 nm (3.14); IR (KBr) ν_{max} (cm^{-1}): 3535, 3445, 3081, 1725, 1716, 1661, 1624, 1489, 1378, 1260, 1147,

1064; ^{1}H and ^{13}C NMR spectral data are shown in Table 1; HR-ESI-MS *m/z*: 469.1818 $[M + Na]^+$ (calcd for $C_{24}H_{30}O_8Na$, 469.1838).

3.4 Cytotoxicity assay

Cytotoxicity of compounds **4**–**9** toward HL60, SMMC-7721, and HeLa cells was determined in 96-well microtiter plates by the sulforhodamine B method [9]. Briefly, exponentially growing HL60, SMMC-7721, and HeLa cells were harvested and seeded in 96-well plates with the final volume 100 μl containing 4×10^3 cells per well for 24 h. Then, using mitomycin as a positive control, cells were treated with various concentrations of compounds (**4**–**9**) for 48 h. The cultures were fixed at 4°C for 1 h by addition of ice-cold 50% trichloroacetic acid to give a final concentration of 10%. Fixed cells were rinsed five times with deionized water and stained for 10 min with 0.4% sulforhodamine B dissolved in 0.1% acetic acid. The wells were washed five times with 0.1% acetic acid and left to dry overnight. The absorbed sulforhodamine B was dissolved in 150 μl unbuffered 1% Tris base [tris(hydroxymethyl)aminomethane] solution in water (pH 10.5). The absorbency of extracted sulforhodamine B at 515 nm was measured on a microplate reader (Bio-Rad, Hercules, CA, USA). The experiments were carried out in triplicate. Each run entailed five to six concentrations of the compounds being tested. The percentage survival rates of cells exposed to the compounds were calculated by assuming the survival rate of untreated cells to be 100%.

References

[1] Jiangshu New Medical College, *Dictionary of Chinese Materia Medica* (Shanghai Scientific and Technical Publishers, Shanghai, 1977), p. 159.
[2] L.M. Li, G.Y. Li, and L.S. Ding, *J. Nat. Prod.* **71**, 684 (2008).
[3] X.R. Wang, H.P. Hu, and H.P. Wang, *Phytochemistry* **37**, 1367 (1994).
[4] E. Fujita, M. Taoka, and M. Shibuya, *Chem. Pharm. Bull.* **21**, 1357 (1973).
[5] J.X. Liu, H.M. Gao, and Z.M. Wang, *Mod. Chin. Med.* **9**, 10 (2007).
[6] T. Fujita, Y. Takeda, T. Shingu, and A. Ueno, *Chem. Lett.* 1635 (1980).
[7] Y.M. Hu, W.C. Ye, and Z.Q. Yin, *Acta Pharm. Sin.* **42**, 286 (2007).
[8] J.H. Chao, Q.Z. Zhao, and H.Q. Wang, *Acta Bot. Yunnan* **5**, 311 (1983).
[9] P. Skehan, R. Stoneng, and D. Scudiero, *J. Natl Cancer Inst.* **82**, 1107 (1990).