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ENT-KAURANE DITERPENOIDS FROM *ISODON RUBESCENS* VAR. *LUSHIENSIS*

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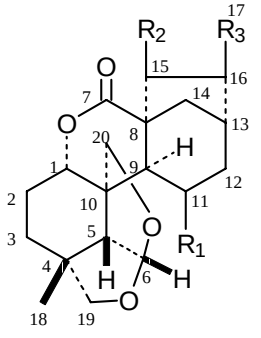
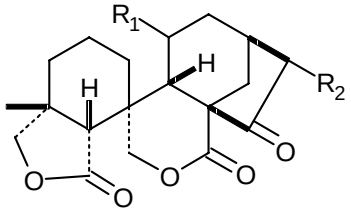
Abstract – Three new 6,7-seco-6,19:6,20-diepoxy-1α,7-olide-*ent*-kaurane diterpenoids, ludongnins C-E (**1**-**3**), along with six known analogs, macrocalyxoformins A (**4**) and B (**5**), sculponeatins A (**6**) and B (**7**), and ludongnins A (**8**) and B (**9**), were isolated from *Isodon rubescens* var. *lushiensis*. The structures of the new compounds were determined on the basis of the spectroscopic evidences. Compound (**5**) exhibited significant inhibitory activities against the Bcap37 and K562 cell lines with IC_{50} = 0.82 and 0.62 μg/mL, respectively.

INTRODUCTION

The plants of the genus *Isodon* (Labiatae) are well known as a rich source of *ent*-kaurane diterpenoids.[1] About twenty *ent*-kaurane diterpenoids have been reported from *Isodon rubescens*,[2] which was the first studied species of this genus plants in China. Among them, oridonin and ponicidin which belong to



	R_1	R_2	R_3
1	H	= O	α-CH_3
2	H	= O	β-CH_3
3	β-OH	= O	α-CH_3
4	H	α-OH	= CH_2
5	H	= O	= CH_2
6	β-OH	= O	= CH_2
7	β-OH	α-OH	= CH_2



	R_1	R_2
8	α-OH	= CH_2
9	H	α-CH_3

7,20-epoxy type *ent*-kauranoids were regarded as the main diterpene constituents of this plant.[3] However, there were only two 6,7-seco-7,20-olide-*ent*-kaurane diterpenoids (ludongnins A and B) reported from *Isodon rubescens* var. *lushiensis* Gao et Li.[4] In our continuing search for biological active principles of *Isodon rubescens* and its varieties, we have reinvestigated on *Isodon rubescens* var. *lushiensis* collected in Lushi Prefecture, Henan Province of China. As a result, three new 6,7-seco-6,19:6,20-diepoxy-1α,7-olide-*ent*-kauranoids named ludongnins C-E (**1**-**3**) together with six known diterpenoids identified as macrocalyxoformins A and B (**4** and **5**),[5] sculponeatins A and B (**6** and **7**) [6], and ludongnins A and B (**8** and **9**) were isolated. In this paper, we wish to present the structural elucidation of these new compounds and the biological testing results of compounds (**5**) and (**7**) against the Bcap37 and K562 tumor cell lines.

RESULTS AND DISCUSSION

Compound (**1**), obtained as colorless quadrate lumpish crystals with $[\alpha]_D^{20}$ –110.0° (*c* 0.82, MeOH), was determined to possess the molecular formula $C_{20}H_{26}O_5$ by the HREIMS (found 346.1762, calcd 346.1780). The ^{13}C and DEPT NMR spectra of **1** showed 20 carbon signals which were indicated to be composed of one carbonyl carbon, one lactonic carbonyl carbon, one acetal carbon, three quaternary carbons, five methines including one oxygenated, five methylenes besides two oxygenated ones, and two methyls, which suggested **1** as an *ent*-kauranoid, combined with the consideration of the structures of diterpenoids previously isolated from this plant.[4] Furthermore, the basic skeleton of 6,7-seco-6,19:6,20-diepoxy-1α,7-olide-*ent*-kaurane was deduced on the basis of the presence of the characteristic signals of a δ-lactone group (δ_C 171.9, C-7; δ_C 76.5, C-1) , two oxymethylene carbons (δ_C 77.0, C-19; δ_C 73.1, C-20), and one acetal group (δ_C 111.3, C-6). Comparison of the ^{13}C NMR spectrum (Table 1) of **1** with that of macrocalyxoformin B (**5**), a known 6,7-seco-6,19:6,20-diepoxy-1,7-olide-*ent*-kauranoid isolated from the same plant this time, revealed that the olefinic signals [δ_C 151.0 (s, C-16) and 118.2 (t, C-17)] in **5** were replaced by a methine signal (δ_C 49.4, C-16) and a methyl signal (δ_C 10.3, C-17) in **1**,

Table 1: ^{13}C NMR Spectral Data (C_5D_5N) of **1**-**4**.

C	**1**	**2**	**3**	**4**
1	76.5 (d)	76.3 (d)	79.1 (d)	76.3 (d)
2	23.0 (t)	23.0 (t)	23.4 (t)	23.2 (t)
3	29.1 (t)	28.9 (t)	29.5 (t)	29.1 (t)
4	41.4 (s)	41.3 (s)	41.7 (s)	41.3 (s)
5	53.0 (d)	53.1 (d)	54.2 (d)	53.3 (d)
6	111.3 (d)	111.3 (d)	111.8 (d)	111.4 (d)
7	171.9 (s)	171.7 (s)	172.3 (s)	171.2 (s)
8	56.6 (s)	56.7 (s)	56.6 (s)	56.5 (s)
9	43.7 (d)	42.9 (d)	47.0 (d)	42.9 (d)
10	50.6 (s)	50.9 (s)	50.4 (s)	50.9 (s)
11	19.2 (t)	19.6 (t)	64.9 (d)	19.3 (t)
12	19.2 (t)	29.3 (t)	30.7 (t)	29.6 (t)
13	32.7 (d)	35.2 (d)	32.9 (d)	35.0 (d)
14	33.9 (t)	32.1 (t)	35.6 (t)	32.5 (t)
15	215.0 (s)	214.9 (s)	215.9 (s)	200.3 (s)
16	49.4 (d)	51.0 (d)	49.8 (d)	151.0 (t)
17	10.3 (q)	15.9 (q)	11.0 (q)	118.0 (t)
18	30.3 (q)	30.3 (q)	30.7 (q)	30.3 (q)
19	77.0 (t)	77.0 (t)	77.2 (t)	77.0 (t)
20	73.1 (t)	72.9 (t)	73.1 (t)	72.8 (t)

which was approved by the ^{1}H-^{1}H COSY correlations of a methine proton (δ_H 2.43, H-16) with a methyl group (δ_H 0.99, H_3-17) and a methine proton (δ_H 2.41, H-13), and by the HMBC correlations (Figure 1) of this methyl group with a ketonic carbon (δ_C 215.0, C-15) and a methine carbon (δ_C 32.7, C-13). The α-orientation of 16-methyl group was determined by the significant upfield shift of C-12 (Δδ 10.4, from δ_C 29.6 in **5** to δ_C 19.2 in **1**) caused by a steric compression effect between Me-16α and H-12α, which was confirmed by the ROESY correlation (Figure 1) between H_3-17 and H-12α. Therefore, compound (**1**) was elucidated as 16(*R**)-6,7-seco-6,19;6,20-diepoxy-1,7-olide-*ent*-kaur-15-one, named ludongnin C.

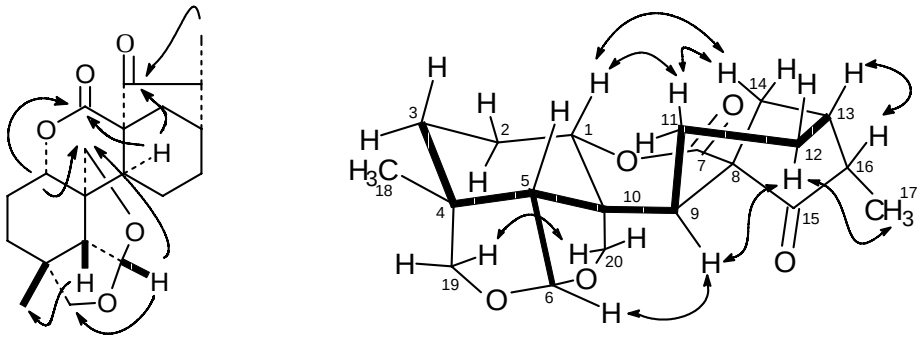


Figure 1 Key HMBC (from H to C) and ROESY Correlations of **1**

Compound (**2**) exhibited the same HREIMS experimental result (found 346.1779, calcd 346.1780 for $C_{20}H_{26}O_5$) as **1**. A general analysis of all spectra obtained revealed that the structure of **2** was quite similar to that of **1** except for two obvious downfield shifts of C-12 and C-17 (Table 1), suggesting **2** to be the 16(S)-epimer of **1** without the above-mentioned steric compression effect. This deduction was proved by the correlations between H_3-17 with H-13β and H-14α in the ROESY spectrum of **2**. Then, compound (**2**) was determined as 16(*S**)-6,7-seco-6,19:6,20-diepoxy-1,7-olide-*ent*-kaur-15-one, named ludongnin D.

Compound (**3**) showed the molecular ion peak at *m/z* 362 in the EIMS spectrum with the molecular formula $C_{20}H_{26}O_6$ determined by the HREIMS (found 362.1747, calcd 362.1729). Comparison of the ^{13}C NMR data of compounds (**1**) and (**3**) suggested that compound (**3**) was consistent with **1** except for a more hydroxyl group at C-11 of **3** which was approved by the HMBC correlations arising from H-11 (δ_H 4.43) with C-10 (δ_C 50.4) and C-13 (δ_C 32.9). The β-orientation of OH-11 was established by the small coupling constant (J = 4.0 Hz) between H-11α with H-9α (δ_H 2.01) and the ROESY correlation between H-11α with H-5β (δ_H 2.96), which was confirmed by the downfield shifts of H-1β (δ_H 5.51) and H-14β (δ_H 3.75),[6] and by the upfield shift of C-11 (δ_C 64.9) caused by the steric compress effect between H-11α

with H-5β. Hence, compound (**3**) was established as 16(*R**)-6,7-seco-11β-hydroxy-6,19:6,20-diepoxy-1α,7-olide-*ent*-kaur-15-one, named ludongnin E.

Six known diterpenoids isolated from *I. rubescens* var. *lushiensis* were identified as macrocalyxoformins A and B (**4** and **5**),[5] sculponeatins A and B (**6** and **7**),[6] and ludongnins A and B (**8** and **9**),[4] respectively, by comparing their spectral data with those reported in the literatures. The ^{13}C NMR spectral data of macrocalyxoformins B (**5**) were reported for the first time. And the chemical shifts of C-19 and C-20 in compounds (**6**) and (**7**) were revised to exchange according to the HMBC correlations between H-19 with C-18, and H-20 with C-1.

Compounds (**5**) and (**7**) were tested for their inhibitory activities against the Bcap37 and K562 cell lines using a previously described method with cis-platinum as a positive reference substance.[7] Comound (**5**) showed significant inhibitory activities against the Bcap37 and K562 cell lines with IC_{50} = 0.82 and 0.62 μg/mL (cis-platinum IC_{50} = 1.54 and 3.84 μg/mL), respectively, while compound (**7**) was inactive against either Bcap37 or K562 cell lines, which confirmed that the cyclopentanone conjugated with an exo-methylene group was the active center of the inhibitory effect.[8]

EXPERIMENTAL

Gerneral: Melting points were measured on an XRC-1 micro melting point apparatus and uncorrected. Optical rotations were taken on a JASCO DIP-370 digital polarimeter. IR spectra were determined on Bio-Rad FtS-135 spectrophotometer with KBr pellets. UV spectra were obtained on a UV 210A spectrophotometer. MS were recorded on a VG Auto Spec-3000 spectrometer. 1D- and 2D- NMR spectra were run on a Brucker AM-400 and a DRX-50 instrument with TMS as internal standard, respectively.

Plant material: The leaves of *I. rubescens* var. *lushiensis* were collected in Lushi prefecture of Henan Province in August 2000, and air-dried. The identity of the plant material was verified by Prof. Zhong-Wen Lin, and a voucher specimen (KIB-2000-10 Lin) is deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Chinese Academy of Science.

Extraction and Isolation: The dried and powdered leaves (7.6 kg) were extracted with 70% Me_2CO (3×10 L) at rt for 72 h and filtered. And then, the filtrate was concentrated and extracted with petroleum ether and EtOAc in turn. The EtOAc extract (350 g) was applied to column chromatography over Silica gel (100-200 mesh, 3.0 kg) column eluting with a gradient system of $CHCl_3$-Me_2CO (1:0-1:1). The $CHCl_3$-Me_2CO (8:2) fraction (40 g) was further chromatographed

repeatedly over Silica gel (cyclohexane: EtOAc = 4:1) to afford **4** (1.0 g), **5** (300 mg), **6** (150 mg), and **7** (120 mg). The $CHCl_3$-Me_2CO (7:3) fraction (23 g) was chromatographed on Silica gel (CH_2Cl_2: 2-propanol, from 150:1 to 25:1), then fined with preparative TLC (developed with CH_2Cl_2: 2-propanol = 100:1 for four times) and recrystallization to yield compounds (**1**) (6.0 mg), (**2**) (2.0 mg), and (**3**) (20.0 mg).

Ludongnin C (1): colorless quadrate lumpish crystals; mp > 250°C; $[\alpha]_D^{20}$ –110.0° (*c* 0.82, MeOH); UV (MeOH): end absorption; IR (KBr) ν_{max}: 3436, 2947, 2875, 1760 (C=O), 1720 (C=O), 1392, 1268, 1195, 1113, 1037 cm^{-1}; ^1H NMR (500 MHz, C_5D_5N): 6.03 (1H, d, *J* = 5.2 Hz, H-6β), 4.64 (1H, dd, *J* = 5.3, 11.6 Hz, H-1β), 4.23 and 4.06 (each 1H, d, *J* = 9.0 Hz, H_2-20), 3.96 and 3.42 (each 1H, d, *J* = 8.7 Hz, H_2-19), 2.59 (1H, d, *J* = 12.0 Hz, H-14β), 2.43 (1H, overlapped, H-16β), 2.41 (1H, overlapped, H-13β), 2.37 (1H, d, *J* = 5.2 Hz, H-5β), 2.10 (1H, overlapped, H-12β), 2.07 (1H, overlapped, H-9α), 1.86 (1H, overlapped, H-14α), 1.86-1.78 (2H, overlapped, H_2-2), 1.72 (1H, m, H-12α), 1.70-1.34 (2H, overlapped, H_2-11), 1.62 and 1.40 (each 1H, overlapped, H_2-3), 1.04 (3H, s, Me-18), 0.99 (3H, d, *J* = 6.9 Hz, Me-17); ^{13}C NMR (C_5D_5N) see Table 1; EIMS *m/z*: 346 $[M]^+$ (32), 328 (36), 318 (40), 300 (20), 289 (44), 272 (18), 258 (32), 165 (77), 136 (42), 121 (39), 107 (60), 91 (65), 55 (100); HREIMS *m/z*: 346.1762 (calcd for $C_{20}H_{26}O_5$, 346.1780).

Ludongnin D (2): white amorphous powder; $[\alpha]_D^{20}$ –60.0° (*c* 0.30, MeOH); UV (MeOH): end absorption; ^1H NMR (500 MHz, C_5D_5N): 6.02 (1H, d, *J* = 5.0 Hz, H-6β), 4.62 (1H, dd, *J* = 5.3, 11.4 Hz, H-1β), 4.21 and 4.04 (each 1H, d, *J* = 9.0 Hz, H_2-20), 3.96 and 3.42 (each 1H, d, *J* = 8.7 Hz, H_2-19), 2.52 (1H, d, *J* = 11.8 Hz, H-14β), 2.50 (1H, overlapped, H-16α), 2.16 (1H, overlapped, H-13β), 2.39 (1H, d, *J* = 5.0 Hz, H-5β), 2.10 (1H, overlapped, H-12α), 2.00 (1H, overlapped, H-12β), 2.11 (1H, overlapped, H-9α), 1.82 (1H, overlapped, H-14α), 1.88-1.77 (2H, overlapped, H_2-2), 1.68 and 1.32 (each 1H, overlapped, H_2-11), 1.60 and 1.39 (each 1H, overlapped, H_2-3), 1.04 (3H, s, Me-18), 1.07 (3H, d, *J* = 6.9 Hz, Me-17); ^{13}C NMR (C_5D_5N), see Table 1; EIMS *m/z*: 346 $[M]^+$ (32), 328 (60), 318 (90), 300 (24), 289 (70), 272 (35), 259 (60), 165 (100), 107 (70), 91 (75), 55 (100); HREIMS *m/z*: 346.1779 (calcd for $C_{20}H_{26}O_5$, 346.1780).

Ludongnin E (3): white amorphous powder; $[\alpha]_D^{20}$ –122.5° (*c* 0.20, acetone); UV (MeOH): end absorption; ^1H NMR (500 MHz, C_5D_5N): 6.11 (1H, d, *J* = 5.0 Hz, H-6β), 5.51 (1H, dd, *J* = 5.5, 12.0 Hz, H-1β), 4.43 (1H, t, *J* = 4.0 Hz, H-11α), 4.26 and 4.11 (each 1H, d, *J* = 10.0 Hz, H_2-20), 4.01 and 3.45 (each 1H, d, *J* = 8.5 Hz, H_2-19), 3.75 (1H, d, *J* = 11.0 Hz, H-14β), 2.59 (1H, m, H-13β), 2.52 (1H, m, H-16β), 2.96 (1H, d, *J* = 5.0 Hz, H-5β), 2.09 (1H, overlapped, H-14α), 2.05 (1H, overlapped, H-12β),

2.01 (1H, d, *J* = 4.0 Hz, H-9α), 1.96-1.74 (2H, overlapped, H_2-2), 1.74 (1H, dd, *J* = 4.0, 15.5 Hz, H-12α), 1.68-1.58 (2H, overlapped, H_2-3), 1.07 (3H, s, Me-18), 1.03 (3H, d, *J* = 7.0 Hz, Me-17); ^{13}C NMR (C_5D_5N), see Table 1; EIMS *m/z*: 362 $[M]^+$ (32), 346 (36), 332 (95), 314 (100), 298 (24), 286 (48), 270 (10), 260 (22), 220 (14), 165 (26), 107 (39), 91 (56), 83 (64), 55 (62); HREIMS *m/z*: 362.1747 (calcd for $C_{20}H_{26}O_6$, 362.1729).

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