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Two novel diterpenoids from *Isodon rubescens* var. *lushanensis*

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ABSTRACT

Two novel diterpenoids, luanchunins A (**1**) and B (**2**), along with their precursor, kamebakaurin (**3**), had been isolated from the stems and leaves of *Isodon rubescens* var. *lushanensis*. Their structures were elucidated on the basis of extensive spectroscopic analyses. Compounds **1** and **2** showed potent cytotoxic activity against HL-60 with IC_{50} values of 4.81 μM and 3.52 μM, respectively. Plausible pathways for the biosynthesis of **1** and **2** were also postulated.

Isodon genus (formerly named *Rabdosia*) is a cosmopolitan and important genus in Labiatae family and consists of about 150 species.[1] Phytochemical studies on this genus led to the isolation of a series of chemically diverse and biologically interesting metabolites.[2] Since 1976, our group have investigated 66 *Isodon* species and found more than 600 new diterpenoids, some of which have antitumor activities.[3] Many novel compounds had been characterized in the past 30 years, such as 1:1 complexes of natural *ent*-kauranoids (Diter-Complex-RA),[4] 6,7:8,15-*seco*-*ent*-kauranoids (laxiflorins F and G),[5] 15,16-*seco*-*ent*-kauranoid (rubescensin S),[6] symmetric and asymmetric *ent*-kauranoid dimers (maoecrystal M,[7] bisrubescensins A-C),[8] and novel C_{19} skeleton maoecrystal V.[9] However, *ent*-kaurane diterpenoids with a seven-membered B-ring or a cleavage of A-ring between C-1 and C-10 have never been reported so far.

In previous studies, *Isodon rubescens* var. *lushanensis*, collected in Lushan Prefecture of Henan Province, five 20-nonoxygenated, and fifteen 20-oxygenated *ent*-kaurane diterpenoids were reported.[10–14] As part of a research for more new diterpenoids with significant antitumor activity, we further explored *I. rubescens* var. *lushanensis* collected in Luanchuan Prefecture. As a result, two novel diterpenoids (**1** and **2**), with a rare seven-membered B-ring and a cleavage in A-ring between C-1 and C-10, respectively, together with their precursor, kamebakaurin (**3**),[15] were obtained. Herein, we report the isolation, structure elucidation, cytotoxicity evaluation, and their proposal biogenetic pathways of two novel diterpenoids.

Compound **1** exhibited a quasi-molecular ion peak at *m/z* 371.1833 ([M+Na]$^+$, calcd 371.1834) in its HR-ESIMS, corresponding to $C_{20}H_{28}O_5$, establishing seven degrees of unsaturation. IR spectrum of **1** showed absorption bands for hydroxyl (3438 cm^{-1}), conjugated ketone (1724 cm^{-1}), and olefinic (1647 cm^{-1}) groups. ^{1}H, ^{13}C NMR, and DEPT-NMR data of compound **1** (Table 1) indicated the presence of one exomethylene, one conjugated carbonyl group, two tertiary methyls, five methylenes, seven methines (including three oxygenated ones), and three quaternary carbons (including one oxygenated). Except for the exomethylene and the ketone, seven degrees of unsaturation of **1** suggested that compound **1** should possess 5 rings in its structure. Typical carbon signals of **1** (δ_C 34.3, C-4; δ_C 63.6, C-8; δ_C 25.4, 28.2, C-18, 19; δ_C 208.2, C-15; δ_C 147.6, 117.1; C-16, 17) demonstrated that it could possess a structure partially similar to that of compound **3**. But one diagnostic quaternary carbon signal of **3** (δ_C 47.9, s, C-10) disappeared in the ^{13}C NMR spectrum of **1**, and one additional methine signal (δ_C 41.5, d) occurred in **1**, which suggested that the B-ring of **1** may be rearranged uncommonly. HMBC correlations arising from ^{1}H signal (δ_H 2.55, d) to C-2 (δ_C 24.5, t), C-4 (δ_C 34.3, s), and C-5 (δ_C 39.9, d) indicated that the ^{1}H signal could be assigned to H-10 as shown in Figure 1. ^{1}H–^{1}H COSY correlations of H-10/H_2-1 confirmed the above attribution. HMBC correlations of H-20 (δ_H 3.99, t) with C-11 (δ_C 21.5, t), C-5 (δ_C 39.9, d), and C-8 (δ_C 63.6, s), revealed that C-20 (δ_C 81.0, d) was connected with C-9 (δ_C 42.3, d) through carbon–carbon bond. This linkage showed that compound **1** possessed a rare expanded seven-membered B-ring. The above deduction was further confirmed by ^{1}H–^{1}H COSY correlations between H-20 (δ_H 3.99, t) and H-9 (δ_H

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Table 1
^{1}H and ^{13}C NMR data of **1** and **2**

Position	**1**[a] (*J* in Hz)		**2**[b] (*J* in Hz)	
	δ_H	δ_C	δ_H	δ_C
1α	1.59[c], m	37.4 (t)	3.82, m	61.5 (t)
1β	1.74[c], m			
2	1.98[c], m	24.5 (t)	1.66[c], m	26.6 (t)
3α	1.11[c], m	30.5 (t)	1.61, m	26.1 (t)
3β	1.66[c], m			
4		34.3 (s)		33.4 (s)
5β	1.52, m	39.9 (d)	1.93[c], m	48.8 (d)
6α		100.2 (s)	1.90[c], m	34.3 (t)
6β			2.47, m	
7α	4.32, d (3.4)	72.0 (d)		71.1 (d)
7β			4.82, br d (11.5)	
8		63.6 (s)		60.1 (s)
9α	2.00[c], m	42.3 (d)		
9β			2.30, d (6.6)	47.1 (d)
10β	2.55, d (5.5)	41.5 (d)		146.7 (s)
11α	1.60[c], m	21.5 (t)	1.63[c], m	19.9 (t)
11β	1.66[c], m		1.53[c], m	
12α	2.28[c], m	31.7 (t)	2.01, m	28.4 (t)
12β	1.77[c], m		1.60[c], m	
13α	3.09, br s	45.1 (d)	3.22, br s	46.5 (d)
14α	4.88, br s	74.8 (d)	4.71, s	74.4 (d)
15		208.2 (s)		205.4 (s)
16		147.6 (s)		148.9 (s)
17a	5.35, ABd (5.4)	117.1 (t)	5.37, s	115.2 (t)
17b	6.05, ABd (5.4)		6.31, s	
18	1.02, s	25.4 (q)	1.07, s	23.6 (q)
19	0.90, s	28.2 (q)	1.03, s	24.9 (q)
20β	3.99, t (5.7)	81.0 (d)		
20a			4.97, s	108.2 (t)
20b			5.30, s	

[a] Data were recorded in $CDCl_3$ + MeOD on Bruker DRX-500 MHz spectrometer.
[b] Data were recorded in C_5D_5N on Bruker AV-400 MHz spectrometer.
[c] Overlapping signals.

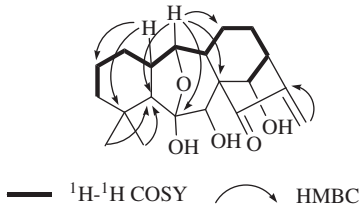


Figure 1. Key ^{1}H–^{1}H COSY and HMBC (from H to C) of compound **1**.

2.00, m, overlap), H-10 (δ_H 2.55, d), observed in **1**. Other key HMBC correlations arising from H-20 (δ_H 3.99, t) to C-5 (δ_C 39.9), C-8 (δ_C 63.6, s), and C-6 (δ_C 100.2, s), along with HMBC correlations from H-7 (δ_H 4.32, d), H-10 (δ_H 2.55, d), and H-5 (δ_H 1.52, m) to C-6, disclosed the fact that C-20 was connected with C-6 through an oxygen atom, which was further confirmed, and excluded from other possible linkage manner by comparing the chemical shift value of C-4, C-10, and C-13 with those of rabdoloxin B.[16] Therefore, compound **1** was elucidated as an *ent*-kaurane diterpenoid with a rearranged 9(10→20)-*abeo* skeleton.

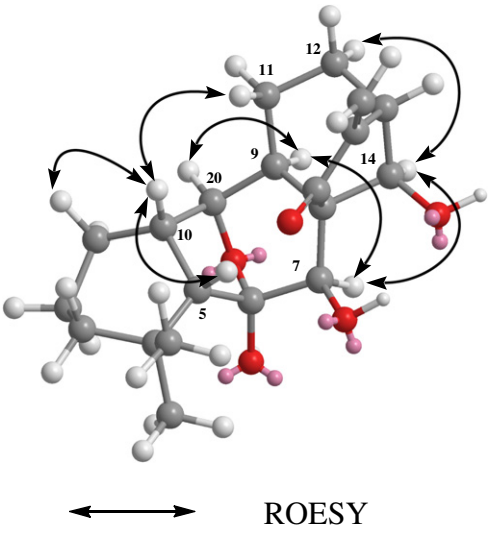
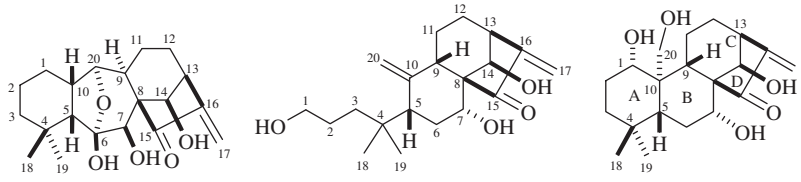


Figure 2. Key ROESY correlations of compound **1**.

The relative stereochemistry of **1** was established by the ROESY experiment (Fig. 2). Considering compound **1** was one biogenetic derivative of *ent*-kaurane diterpenoid, and the great changes of molecular structure occurred at C-10 and C-9, the stereochemistry of C-5 was thought to be retained. ^{1}H–^{1}H correlation H-5/H-10 indicated β-orientation of H-10. H-14 adopted α-orientation, which was based on the correlations H-14/H-12α and H-14/H-13α. The α-orientation of H-7 was established by correlation H-7/H-14α. Correlations of H-7α with H-9 gave an α-orientation of H-9. The α-oriented oxygen bridge between C-20 and C-6 was determined by the correlations between H-20/H-9α, H-10β/H-11β, and H-10β/H-1β, observed in the ROESY spectrum of **1**. Consequently, compound **1** was determined to be 6β,7β,14β-trihydroxy-9(10→20)-*abeo*-6,20-epoxy-*ent*-kaur-16-en-15-one.

Compound **2** showed a quasi-molecular ion peak at *m/z* 357.2007 ([M+Na]$^+$, calcd 357.2041) in its HR-ESIMS, corresponding to $C_{20}H_{30}O_4$, requiring six degrees of unsaturation. IR spectrum of **2** showed absorption bands for hydroxyl (3422 cm^{-1}), conjugated ketone (1728 cm^{-1}), and olefinic (1647 cm^{-1}) groups. Only 16 carbon signals (including C-10, δ_C 146.7, s; overlapping with the solvent signal) were recorded in the ^{13}C NMR spectrum of compound **2** (Table 1). There were two obvious methyl singlets (δ_H 1.03, s, H_3-19; δ_H 1.07, s, H_3-18) in ^{1}H NMR spectrum. But in DEPT spectrum, their corresponding carbon signals could not be detected. HSQC correlations of two methyls (δ_H 1.03; δ_H 1.07) with ^{13}C-signals (hidden, δ_C 24.9; δ_C 23.6) testified that C-19 and C-18 actually did not resonate in their appropriate frequency in ^{13}C NMR spectrum. So did the methine (hidden, δ_C 48.8, d, C-5) and one methylene (hidden, δ_C 26.1, t, C-3) in the HSQC spectrum. Finally, compound **2** was found to possess two tertiary methyls, eight methylenes (including two exomethylenes), five methines, and five quaternary carbons. Typical carbon signals suggested that **2** may also possess a skeleton partially similar to compound **3**. Comparing with **3**, two important carbon signals of **3** (C-10, C-20) could not be found in **2**, while two corresponding carbon signals (C-10, δ_C 146.7, s; C-20, δ_C 108.2, t) occurred in the olefinic re-



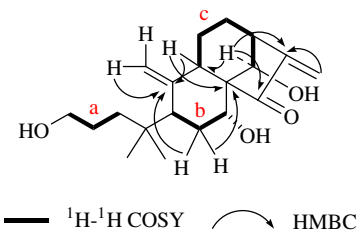


Figure 3. Key ^{1}H–^{1}H COSY and HMBC (from H to C) of compound **2**.

gion of **2**. And the exomethylene (δ_C 108.2, t, C-20) was located at C-10 as there were HMBC correlations arising from H-20a (δ_H 4.97, s), H-9 (δ_H 2.30, d), and H-6 (δ_H 2.47, m) to C-10 (δ_C 146.7, s). Considering six degrees of unsaturation (including two exomethylenes and one ketone) of **2**, it means that compound **2** only possess 3 rings in its structure. The exomethylene located at C-10 showed that compound **2** possessed one opening A-ring (cleavage in the bond C-1/C-10 of **3**) derived from the *ent*-kaurane skeleton comparing with four rings of **3**. All the above deductions were further confirmed by ^{1}H–^{1}H COSY spectrum. Partial structures **a** (H-1 to H-3), **b** (H-5 to H-7), and **c** (H-9 to H-14) were clearly observed in COSY spectrum as shown in Figure 3.

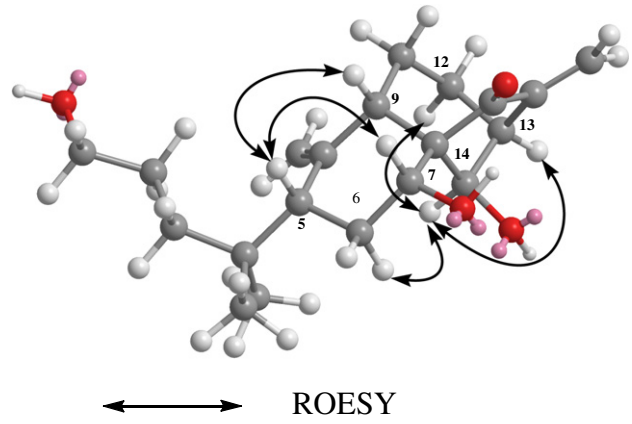
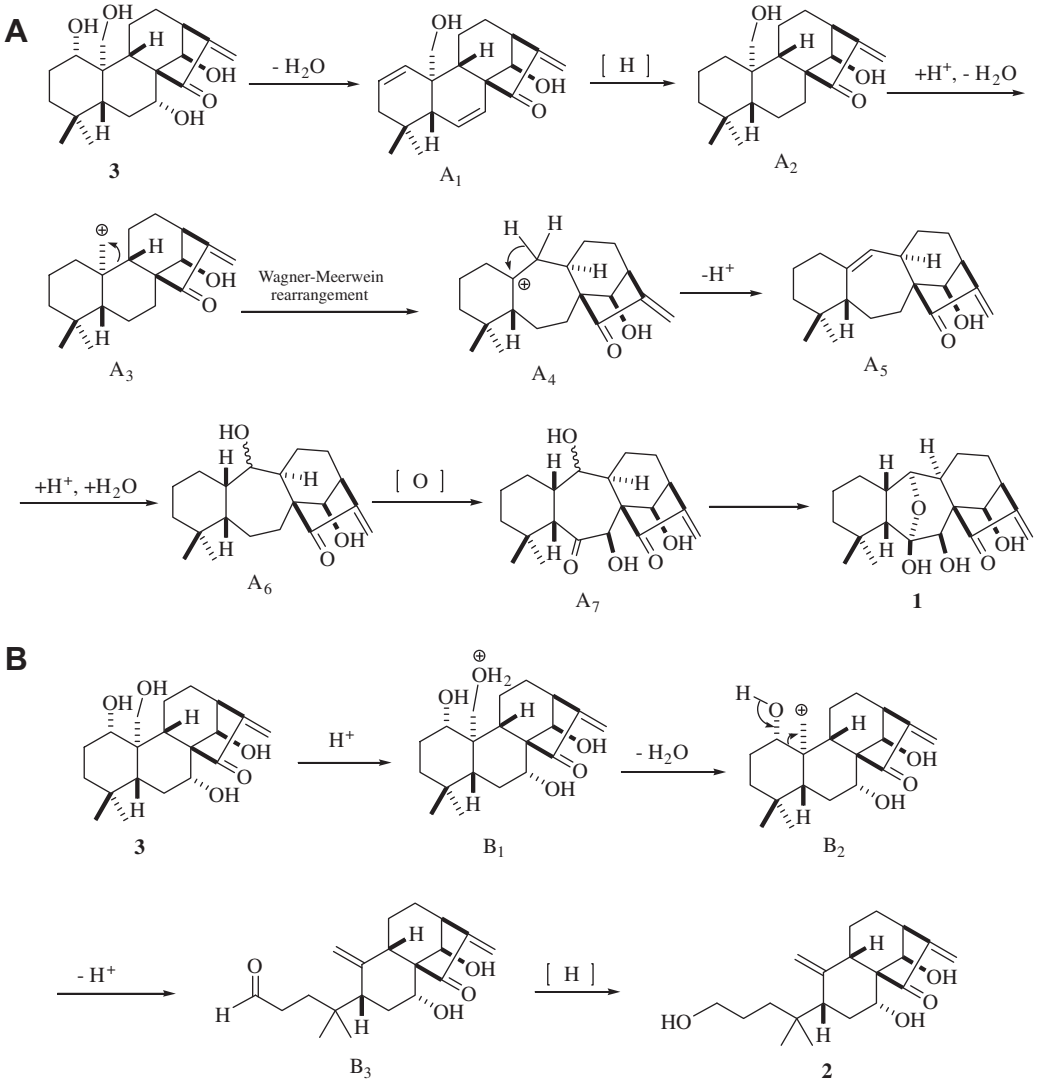


Figure 4. Key ROESY correlations of compound **2**.

ROESY spectrum revealed the relative stereochemistry of C-5, C-7, and C-14 of **2** (Fig. 4). Considering compound **2** was also one biogenetic derivative from *ent*-kaurane diterpenoid, and the great change of structure occured in ring A, the stereochemistry of C-9 was thought to be retained. Correlations between H-5, H-7, and H-9β indicated that H-5 and H-7 possess β-orientations,



Scheme 1. Proposed biogenetic pathway for compounds **1** (A) and **2** (B).

Table 2
Cytotoxicity data of **1** and **2**

Compounds	IC_{50} (μM) for cell lines				
	HL-60	SMMC-7721	A-549	PANC-1	SK-BR-3
1	4.81	24.62	>40	37.46	30.09
2	3.52	>40	>40	29.83	16.58
cis-Platin	1.67	19.36	29.70	17.38	37.97

respectively. And correlations of H-13α, H-12α, and H-6α with H-14 indicated that H-14 possessed α-orientation. Consequently, compound **2** was determined as 1,7α,14β-trihydroxy-1,10-*seco*-*ent*-kaur-10,16-dien-15-one.

A biogenetic pathway was proposed for compounds **1** and **2** (Scheme 1). Compound **3** was taken as the common precursor for **1** and **2**. For **1**, the key reaction happened in the step from A_3 to A_4. Cleavage took place in the bond of C-9/C-10, then C-9 (with configuration reversed) was rearranged to C-20 resulting in the form of A_4. This rearrangement once was reported in one similar structure by one reference.[17] After oxidization at C-6, C-7 (A_7), then by condensation with the hydroxyl group at C-20, compound **1** was formed finally. As for compound **2**, the key cleavage appeared in bond C-1/C-10 resulting in the form of B_3. This cleavage had been proved by one reference.[18] Further hydrogenation at the aldehyde group of B_3 produced compound **2**. The general feature of the above two biogenetic pathways was that C-20 of **3** was ionized at first, then the vicinal carbon bonds (C-9/C-10 or C-1/C-10) happened broken to produce compounds **1** and **2**.

Compounds **1** and **2** were evaluated for their cytotoxicity in five human tumor cell lines (HL-60, SMMC-7721, A-549, PANC-1, and SK-BR-3). Results were presented in Table 2. Compounds **1** and **2** showed potent cytotoxicity with IC_{50} of 4.81 μM and 3.52 μM in HL-60 cell lines, respectively.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.06.015.

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