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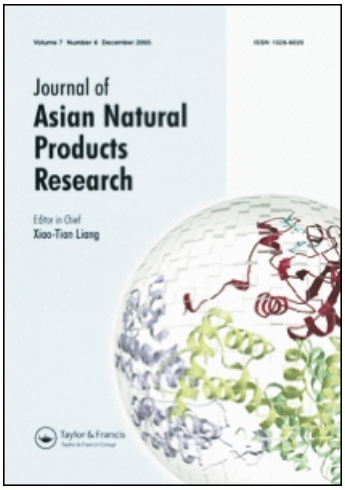

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Ent-kauranoids from *Isodon rubescens* var. *taihangensis*

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Two new compounds, rubescensins Q and R (**1** and **2**), and a new acetonide derivative (**3**) of lasiodonin, together with thirteen known analogues, oridonin (**4**), ponicidin (**5**), wikstroemioidin B (**6**), lasiodonin (**7**), lasiokaurin (**8**), enmenol (**9**), 1-*O*-β-D-glucopyranosyl-enmenol (**10**), trichokaurin (**11**), the acetonide of maoyecrystal F (**12**), rabdoternins A–D (**13**–**16**), have been isolated from *Isodon rubescens* var. *taihangensis*. The structures of the new compounds were elucidated on the basis of spectroscopic methods, especially the 2D NMR spectral analysis. Compound **3** exhibited cytotoxicity against K562, Bcap37, CA, CNE, BIU87, BGC823, and HeLa cell lines.

Keywords: *Isodon rubescens* var. *taihangensis*; Labiatae; *ent*-Kaurene diterpenoid; Rubescensins Q and R; Cytotoxic activity

1. Introduction

In our on-going search for bioactive diterpenoids from *Isodon rubescens* (Labiatae) and its varieties [1–5], our investigation on the chemical constituents of *Isodon rubescens* var. *taihangensis* has led to the isolation of sixteen diterpenoids, including the two new 7,20-epoxy-*ent*-kaurenoids rubescensins Q and R (**1** and **2**), and a new acetonide derivative (**3**) of lasiodonin. The structures of the new compounds were elucidated on the basis of spectroscopic methods, especially 2D NMR spectral analysis. All compounds were assayed for their cytotoxicity against K562 cells, but only **3**–**5**, **7** and **8** exhibited inhibitory effects. Compound **3** also showed cytotoxicity against Bcap37, CA, CNE, BIU87, BGC823, and HeLa cell lines. We report here the isolation, structural elucidation, and bioassay results for these diterpenoids. The ^{13}C NMR data of **11** are reported for the first time.

2. Results and discussion

After repeated chromatographic purification on silica gel, the EtOAc-soluble portion of the Me_2CO extract of *Isodon rubescens* var. *taihangensis* yielded two new diterpenoids,

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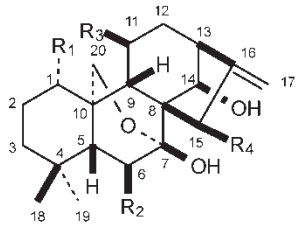
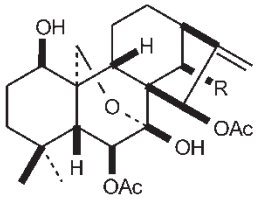
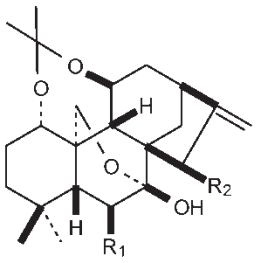
rubescensins Q and R (**1** and **2**), a new acetonide derivative (**3**) of lasiodonin, together with thirteen known ones, oridonin (**4**), ponicidin (**5**), wikstroemioidin B (**6**), lasiodonin (**7**), lasiokaurin (**8**), enmenol (**9**), 1-*O*-β-D-glucopyranosyl-enmenol (**10**), trichokaurin (**11**), the acetonide of maoyecrystal F (**12**), and rabdoternins A–D (**13**–**16**).



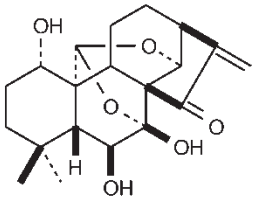
	R_1	R_2	R_3	R_4
1	H	OAc	H	OH
9	OH	OH	H	OH
10	Oglu	OH	H	OH
15	H	OAc	H	OAc
16	H	OAc	OH	OH



	R
2	OH
11	H



	R_1	R_2
3	OH	=O
6	OH	OH
12	OAc	OH



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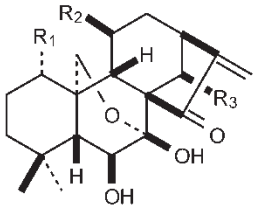
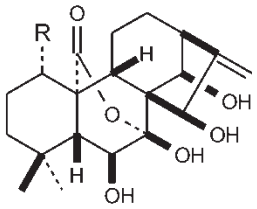


	R_1	R_2	R_3
4	OH	H	OH
7	OH	OH	H
8	OAc	H	OH



	R
13	OH
14	H

Compound **1** exhibited a molecular ion peak at *m/z* 392.2184 in its HR-EIMS, which is consistent with a molecular formula $C_{22}H_{32}O_6$. In the ^{1}H, ^{13}C and DEPT NMR spectra, in addition to the signals of an acetoxy group at δ 169.1 (s), 21.4 (q), 2.16 (3H, s), there were twenty carbon signals which consist of two tertiary methyls, seven methylenes (including one olefinic carbon at δ 110.3 (t) and one oxygenated at δ 66.0 (t)), six methines (including three oxygenated at δ 72.9 (d), 74.3 (d), and 76.0 (d)), and five quaternary carbons (including one hemiketalic carbon at δ 98.4 (s) and one olefinic quaternary carbon at δ 160.7 (s)), suggesting **1** to be a 7,20-epoxy-*ent*-kaurenoid by comparison with reported ^{13}C NMR spectral data [1,2,4]. Comparison of the ^{1}H and ^{13}C NMR spectral data of **1** with rabdoternin C (**15**), a known 7,20-epoxy-*ent*-kaurenoid also isolated from this plant, revealed that **1** was 15-deacetyl-rabdoternin C, which was confirmed by HMBC and ROESY experimental results (figure 1). The OH-15β was determined by the HMBC correlations of H-15 (δ 5.48, d, J = 2.4 Hz) with C-7, C-9 and C-17, and the key ROESY correlation of OH-15 (δ 4.29, d, J = 2.4 Hz) with H-9β (δ 2.60, dd, J = 6.0, 8.8 Hz). Therefore, compound **1** was elucidated to be 7β,14β,15β-trihydroxy-6β-acetoxy-7α,20-epoxy-*ent*-kaur-16-ene, and called rubescensin Q.

Compound **2** has a molecular formula $C_{24}H_{34}O_8$, as determined by the molecular ion peak at *m/z* 450.2239 in the HR-EIMS spectrum. Comparison of the ^{1}H and ^{13}C NMR data of **2**

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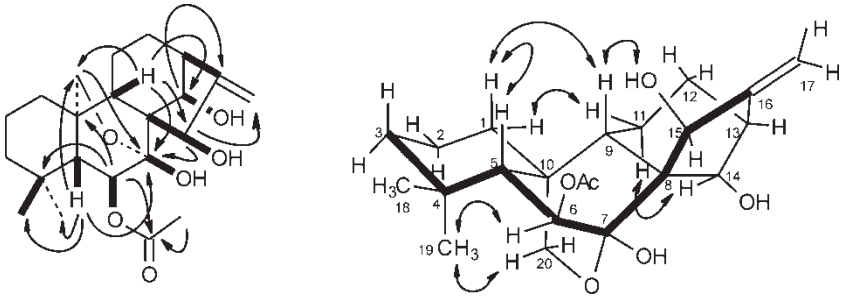

FIGURE 1. Key HMBC and ROESY correlations of **1**.

with those of compounds **1** and **15** disclosed that **2** might be 1β-hydroxy- or 11β-hydroxy-rabdoternin C. Similar comparison with trichokaurin (**11**) further indicated **2** as 14-hydroxytrichokaurin [6]. Furthermore, the OH-1β was determined by the HMBC correlations (figure 2) of H-1 (δ 3.67, br s) with C-5 (δ 49.6, d) and C-20 (δ 66.0, t), and the key ROESY correlation (figure 2) of H-1 with H-11β (δ 1.99, m). Moreover, an acetoxy group was placed at C-15 by the HMBC correlations of H-15 (δ 6.72, s) with C-17 (δ 111.1, t) and the carbonyl carbon of an acetoxy group, being β-orientated, as deduced from the significant upfield signal of C-9 (δ 41.1, d) which was caused by the γ-steric compression between H-9β (δ 3.39, dd, $J = 4.0$, 10.0 Hz) and the 15β-acetoxy group [1]. Thus, compound **2** was assigned as 1β,7β,14β-trihydroxy-6β,15β-diacetoxy-7α,20-epoxy-*ent*-kaur-16-ene, which was confirmed by the HMBC correlations of H-6 (δ 5.97, 1H, d, $J = 6.0$ Hz) with C-4, C-10 and the carbonyl carbon of the other acetoxy group, H_2-20 (δ 4.22 and 4.12, each 1H, d, $J = 8.0$ Hz) with C-7, H-14 (δ 5.09, s) with C-7 and C-16; and the ROESY correlations of H-6α with Me-19 (δ 1.27, s), and H-14α with H-11α (δ 1.39, m).

Similarly, and on the basis of the comparison of the 1D NMR data of **3** and lasiodonin (**7**, one of the major diterpenoids of this plant), **3** was determined as 6β,7β-dihydroxy-7α,20-epoxy-*ent*-kaur-16-en-1α,11β-acetonide, which was confirmed by the HMBC correlations between H-1 and H-11 with the ketalic carbon (δ 101.0, s) of the acetonide group. Compound **3** is probably an artifact of isolation.

All the compounds were assayed for their cytotoxicity against K562 cells using a previously reported method [4], but only compounds **3–5, 7** and **8** exhibited inhibitory activity, with $IC_{50} = 0.01$, 0.14, 1.47, 1.03, and 0.10 μg ml^{-1} (with cisplatin as positive reference, $IC_{50} = 1.14$ μg ml^{-1}), respectively. Compound **3** was further tested for its cytotoxicity against Bcap37 (human breast cancer cell line), CA (human liver cancer cell line), CNE (human nasopharyngeal cancer cell line), BIU87 (human cystic cancer cell line),

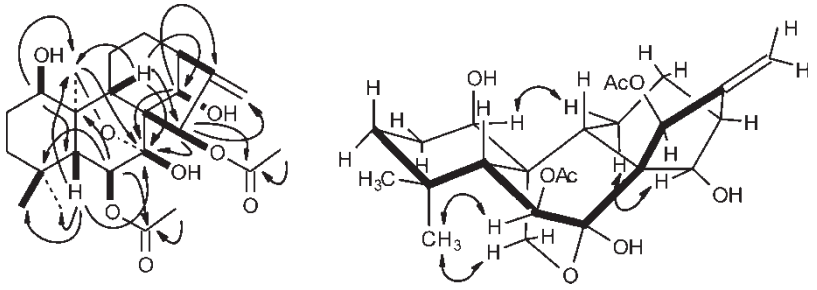

FIGURE 2. Key HMBC and ROESY correlations of **2**.

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Table 1. ^{13}C NMR data of **1–3** and **11** in C_5D_5N (100 MHz, δ in ppm).

	1	*2*	*3*	*11*
1	32.3 (CH_2)	65.5 (CH)	74.3 (CH)	65.5 (CH)
2	18.9 (CH_2)	27.6 (CH_2)	26.5 (CH_2)	27.9 (CH_2)
3	41.1 (CH_2)	34.2 (CH_2)	40.0 (CH_2)	34.4 (CH_2)
4	33.8 (C)	33.9 (C)	33.8 (C)	33.9 (C)
5	55.3 (CH)	49.6 (CH)	60.3 (CH)	50.1 (CH)
6	74.3 (CH)	74.3 (CH)	73.6 (CH)	75.6 (CH)
7	98.4 (C)	98.4 (C)	96.0 (C)	95.9 (C)
8	53.1 (C)	52.0 (C)	59.5 (C)	51.3 (C)
9	43.8 (CH)	41.1 (CH)	54.4 (CH)	39.0 (CH)
10	36.2 (C)	40.7 (C)	40.3 (C)	41.0 (C)
11	15.0 (CH_2)	14.6 (CH_2)	63.5 (CH)	15.4 (CH_2)
12	30.9 (CH_2)	32.0 (CH_2)	37.0 (CH_2)	27.6 (CH_2)
13	45.7 (CH)	45.7 (CH)	34.8 (CH_2)	37.1 (CH)
14	76.0 (CH)	75.9 (CH)	26.8 (CH_2)	32.0 (CH)
15	72.9 (CH)	74.4 (CH)	209.1 (C)	75.6 (CH)
16	160.7 (C)	159.1 (C)	153.2 (C)	160.0 (C)
17	110.3 (CH_2)	111.1 (CH_2)	117.3 (CH_2)	108.9 (CH_2)
18	32.1 (CH_3)	33.2 (CH_3)	33.0 (CH_3)	33.3 (CH_3)
19	22.2 (CH_3)	23.7 (CH_3)	22.5 (CH_3)	23.8 (CH_3)
20	66.0 (CH_2)	66.5 (CH_2)	64.5 (CH_2)	66.5 (CH_2)
OAc	169.1 (C)	171.3 (C)		171.7 (C)
	21.4 (CH_3)	170.8 (C)		171.7 (C)
		22.0 (CH_3)		22.2 (CH_3)
		21.4 (CH_3)		21.6 (CH_3)
Acetonide			101.0 (C)	
			24.6 (CH_3)	
			24.4 (CH_3)	

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BGC823 (human stomach cancer cell line), and HeLa cell lines, with $IC_{50} = 0.37, 0.40, 0.36, 0.01, 0.70$, and $0.08\,\mu g\,ml^{-1}$ in turn (also with cisplatin as positive reference, $IC_{50} = 1.54, 2.54, 4.34, 0.88, 6.54$, and $3.60\,\mu g\,ml^{-1}$). It seemed that the acetonide group of compound **3** contributed much to the cytotoxicity. In addition, the ^{13}C NMR data (table 1) of **11** are reported for the first time.

The diterpenoids all possessed a 7,20-epoxy-*ent*-kaurene skeleton similar to that of diterpenoids isolated from the plant collected in Jiyuan prefecture of Henan Province, and so the major diterpene constituents of the two plant materials collected in Hebi and Jiyuan prefectures are almost identical, and both plants are *Isodon rubescens* var. *taihangensis*, in agreement with the proposed taxonomy [7].

3. Experimental

3.1 General experimental procedures

Melting points were measured on an XRC-1 micro melting apparatus and are uncorrected. IR and UV spectra were obtained on a Bio-Rad FTS-135 infrared spectrometer with KBr pellets and a Shimadzu double-beam 210A spectrometer in MeOH, respectively. Optical rotations were taken on a SEPA-300 polarimeter. The MS spectra were recorded on a VG Autospec-3000 spectrometer (70 eV). ^1H, ^{13}C and 2D NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers with TMS as internal standard. Silica gel for TLC and column chromatography was obtained from Qingdao Marine Chemical Inc., China.

3.2 Plant material

Leaves of *Isodon rubescens* var. *taihangensis* Z. Y. Gao et Y. R. Li were collected in Hebi Prefecture, Henan Province, China, in August 2000, and were identified by Professor Z. W. Lin, Kunming Institute of Botany. A voucher specimen has been deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and isolation

The dried and powdered leaves (10 kg) were extracted with 70% Me_2CO (15 L × 3) and filtered. The filtrate was then concentrated and extracted successively with light petroleum and EtOAc. The EtOAc extract (400 g) was applied to column chromatography over a silica gel (100–200 mesh, 3.0 kg) column eluting with a gradient system of $CHCl_3$–MeOH (1:0, 10:1, 9:1,8:2, 7:3) to yield fractions I–X monitored by TLC. Fraction I (60 g) afforded **13** (200 mg), **14** (100 mg), **6** (307 mg), and **1** (15 mg) after being chromatographed over silica gel column eluting with CH_2Cl_2–propan-2-ol (100:1). Fraction II (162 g) was subjected to column chromatography over silica gel eluting with $CHCl_3$–MeOH (50:1) to yield six further fractions (Frs. 1–6). Compounds **12** (124 mg), **11** (152 mg), **15** (60 mg), **16** (30 mg), and **3** (312 mg) were isolated from Fr. 1 by column chromatography over silica gel ($CHCl_3$–MeOH, 50:1). In the same way, compounds **5** (5.1 g), **2** (74 mg), **8** (114 mg) and **3** (15 mg) were successively obtained from Fr. 2, and compounds **7** (680 mg) and **4** (10.2 g) from Fr. 3. Compounds **9** (69 mg) and **10** (133 mg) were obtained from fraction IV by similar column chromatography over silica gel and recrystallization.

Rubescensin Q (**1**): Colorless needles, mp 235–236°C; $[\alpha]_D^{21.9}$ −1.8 (acetone, *c* 0.274); UV (MeOH) λ_{max} (log ε): 205 (2.86) nm; IR (KBr) ν_{max} (cm^{-1}): 3519, 3472, 2950, 2923, 2885, 1751, 1372, 1213, 1170, 1057, 1025, 979; EIMS (70 eV) *m/z* (rel. int.): 392 $[M]^+$ (1), 374 $[M-H_2O]^+$ (4), 332 $[M-AcOH]^+$ (40), 314 $[M-AcOH-H_2O]^+$ (46), 303 (10), 296 (15), 286 (31), 151 (100); HR-EIMS *m/z*: 392.2184 (calcd. for $C_{22}H_{32}O_6$, 392.2199); ^1H NMR (400 MHz, C_5D_5N) δ: 8.15 (1H, s, OH-7β), 7.89 (1H, s, OH-14β), 5.75 (1H, d, $J = 6.4$ Hz, H-6α), 5.65 (1H, s, H-17a), 5.48 (1H, d, $J = 2.4$ Hz, H-15α), 5.35 (1H, s, H-17b), 4.90 (1H, s, H-14α), 4.29 (1H, d, $J = 2.4$ Hz, OH-15β), 4.14 (1H, d, $J = 9.6$ Hz, H-20a), 3.96 (1H, d, $J = 9.6$ Hz, H-20b), 2.78 (1H, d, $J = 9.0$ Hz, H-13α), 2.60 (1H, dd, $J = 6.0, 8.8$ Hz, H-9β), 2.27 (1H, m, H-12α), 2.16 (3H, s, OAc), 1.64 (1H, m, H-12β), 1.56 (1H, d, $J = 6.4$ Hz, H-5β), 1.39 (1H, m, H-11α), 1.27 (1H, overlap, H-3α), 1.22 (2H, overlap, H_2-2), 1.19 (1H, overlap, H-1α), 1.12 (1H, overlap, H-11β), 1.10 (3H, s, Me-19), 1.08 (1H, m, H-3β), 0.90 (1H, overlap, H-1β), 0.88 (3H, s, Me-18); ^{13}C NMR spectral data see table 1.

Rubescensin R (**2**): Colorless needles, mp 241–242°C; $[\alpha]_D^{22.0}$ −87.2 (acetone, *c* 0.172); UV (MeOH) λ_{max} (log ε): 204 (2.83) nm; IR (KBr) ν_{max} (cm^{-1}): 3460, 3342, 2945, 2868, 1735, 1716, 1373, 1262, 1032; EIMS (70 eV) *m/z* (rel. int.): 450 $[M]^+$ (11), 432 $[M-H_2O]^+$ (15), 390 $[M-AcOH]^+$ (100), 372 $[M-AcOH-H_2O]^+$ (13), 362 (4), 348 (15), 330 $[M-2\times AcOH]^+$ (31), 320 (16), 312 (30), 302 (75); HR-EIMS *m/z*: 450.2239 (calcd. for $C_{24}H_{34}O_8$, 450.2254); ^1H NMR (400 MHz, C_5D_5N) δ ppm: 8.36 (1H, s, OH-7β), 7.99 (1H, s, OH-14β), 6.72 (1H, s, H-15α), 5.99 (1H, d, $J = 2.4$ Hz, OH-1β), 5.97 (1H, d, $J = 6.0$ Hz, H-6α), 5.40 (1H, s, H-17a), 5.22 (1H, s, H-17b), 5.09 (1H, s, H-14α), 4.22 (1H, d, $J = 8.0$ Hz, H-20a), 4.12 (1H, d, $J = 8.0$ Hz, H-20b), 3.67 (1H, s, H-1α), 3.39 (1H, dd, $J = 4.0, 10.0$ Hz, H-9β), 2.78 (1H, d, $J = 7.6$ Hz, H-13α), 2.32 (1H, d, $J = 6.0$ Hz, H-5β), 2.28 (2H, overlap, H-3α

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and H-12α), 2.20 (3H, s, OAc), 2.14 (3H, s, OAc), 1.99 (1H, m, H-11β), 1.75 (1H, m, H-2α), 1.39 (1H, m, H-11α), 1.67 (3H, overlap, H-2β, H-11α and H-12β), 1.27 (3H, s, Me-19), 1.08 (1H, br d, $J = 10.6$ Hz, H-3β), 1.05 (3H, s, Me-18); ^{13}C NMR spectral data see table 1.

Acetonide of lasiodonin (**3**): A white amorphous powder; $[\alpha]_D^{25.6}$ −91.4 (MeOH, *c* 0.186); UV (MeOH) λ_{max} (log ε): 237 (3.96) nm; IR (KBr) ν_{max} (cm^{-1}): 3368, 2981, 2949, 2872, 1712, 1641, 1221, 1212, 1168, 1076, 1024; EIMS (70 eV) *m/z* (rel. int.): 404 $[M]^+$ (100), 346 (80), 328 (13), 298 (35), 271 (44), 242 (22); HR-EIMS *m/z*: 404.2182 (calcd. for $C_{23}H_{32}O_6$, 404.2199); ^{1}H NMR (400 MHz, C_5D_5N) δ (ppm): 8.90 (1H, s, OH-7β), 6.87 (1H, d, $J = 10.4$ Hz, OH-6β), 6.01 (1H, s, H-17a), 5.34 (1H, s, H-17b), 4.58 (1H, dd, $J = 10.0$, 18.4 Hz, H-11α), 4.49 (1H, d, $J = 10.0$ Hz, H-20a), 4.12 (1H, d, $J = 10.0$ Hz, H-20b), 4.21 (1H, dd, $J = 5.6$, 10.4 Hz, H-6α), 3.72 (1H, dd, $J = 4.4$, 12.4 Hz, H-1β), 3.04 (1H, dd, $J = 4.0$, 9.8 Hz, H-13α), 2.54 (1H, m, H-12α), 2.46 (1H, br d, $J = 12.6$ Hz, H-14α), 2.38 (1H, dd, $J = 4.0$, 12.6 Hz, H-14β), 1.71 (1H, m, H-2α), 1.57 (1H, m, H-12β), 1.51 (2H, overlap, H-2β and H-9β), 1.46 (1H, m, H-3α), 1.40 (1H, d, $J = 5.6$ Hz, H-5β), 1.34 (6H, s, 2 × Me of acetonide), 1.29 (1H, m, H-3β), 1.21 (3H, s, Me-18), and 1.06 (3H, s, Me-19); ^{13}C NMR spectral data see table 1.

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