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ent-Kaurene diterpenoids from *Isodon oresbius*

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Abstract

Three new *ent*-kaurene diterpenoids, oreskaurins A–C (**1**–**3**), together with ten known *ent*-kaurene diterpenoids, enmenin monoacetate (**4**), effusanin E (**5**), adenolin B (**6**), maoecrystal G (**7**), enmelol (**8**), trichokaurin (**9**), sodoponin (**10**), trichorabdal A (**11**), nodosin (**12**), enmein (**13**), and a flavonoid, vitexin (**14**), were isolated from *Isodon oresbius*. Their structures were determined by spectroscopic means. Compound **12** showed inhibitory activity toward K562 cells with $IC_{50} = 1.43$ μg/ml.

Keywords: *Isodon oresbius*; Labiatae; *ent*-Kaurenoid; Oreskaurins A–C; Cytotoxicity

1. Introduction

A perennial plant, *Isodon oresbius* (W.W. Smith) Kudo (Labiatae), which has been used in Chinese traditional folk medicine to treat internal hemorrhage of the viscus (Wu and Li, 1977), is distributed in the drift of rocks or in thickets at 2100–3400 m of Yunnan, Sichuan and Tibet Provinces, PR China. In previous investigations, some compounds have been reported (Huang et al., 1996), which include one *ent*-kaurenoid (Huang et al., 1999). In our ongoing search for bioactive diterpenoids from the *Isodon* genus (Li, 1988) plants, the reinvestigation on the chemical constituents of *I. oresbius*, which was collected in Zhongdian County of Yunnan Province, led to the isolation of thirteen diterpenoids including three new *ent*-kaurene diterpenoids, namely oreskaurins A–C (**1**–**3**), along with ten known *ent*-kaurenoids, enmenin monoacetate (**4**), effusanin E (**5**), adenolin B (**6**), maoecrystal G (**7**), enmelol (**8**), trichokaurin (**9**), sodoponin (**10**), trichorabdal A (**11**), nodosin (**12**), enmein (**13**), and a flavonoid, vitexin (**14**). Compounds **2** and **12** were tested for their cytotoxicity toward K562 cells. In this paper, we present the isolation and structural elucidation of these new compounds by spectral analysis.

2. Results and discussion

A 70% aq. acetone extract prepared from aerial parts of *I. oresbius* was partitioned between EtOAc and water. The EtOAc layer was subjected repeatedly to column chromatography on resin and silica gel to afford three new compounds, oreskaurins A–C (**1**–**3**), as well as eleven known compounds (**4**–**14**).

Oreskaurin A (**1**) was obtained as a white amorphous powder, whose molecular formula was determined as $C_{22}H_{28}O_8$ by the HR-FABMS (m/z 421.1835 $[M+H]^+$, calcd. 421.1862). The ^{13}C and DEPT NMR spectra of **1** exhibited 20 carbon signals, besides an acetoxyl group at δ_C 169.8 (s) and 20.5 (q), which indicated a methyl, seven methylenes including two oxygenated ones and an olefinic methylene, five methines including two oxymethines, an acetal carbon, three quaternary carbons, and two carbonyl carbons. Considering the structures of the diterpenoids isolated from this plant, along with the characteristic lactonic carbonyl signal at δ_C 170.8 (s) due to C-7 and two oxymethylenes [one at δ_C 78.3 (t) and δ_H 3.95/3.47 (d, $J = 8.0$ Hz) attributable to C-19/H_2-19, and the other resonating at δ_C 71.0 (t) and δ_H 5.40/4.73 (d, $J = 10.8$ Hz) assignable to C-20/H_2-20], compound **1**

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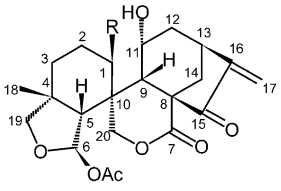
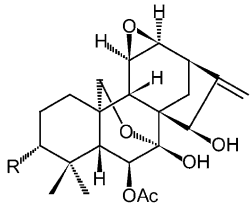
should be a 6,7-*seco*-6,19-epoxy-7,20-olide-*ent*-kaurene diterpenoid similar to trichorabdal F acetate (**16**) (Node et al., 1989). Compound **1** differed from **16** in possessing one more hydroxyl group. Comparison of their ^{1}H and ^{13}C NMR spectra indicated that the additional hydroxyl group was at the C-1 position in **1**. The correlations of H-1 (δ_H 5.96, br s) with C-3 (δ_C 26.0, t), C-5 (δ_C 54.4, d), C-10 (δ_C 40.1, s) and C-9 (δ_C 43.7, d) in the HMBC spectrum of **1** confirmed that the hydroxyl group was located at C-1 and the relative configuration of OH-1β was revealed by a ROESY correlation of H-1α (δ_H 5.96) with H-20 (δ_H 4.73, *d*) (Fig. 1); this was also supported by the upfield shifted C-3 (δ_C 26.0) of **1** comparing with that (δ_C 35.3) of **16** and 6-epiangustifolin (Na et al., 2002) due to the γ-gauche steric compression effect between the OH-1β and H-3β. Therefore, compound **1** was 1β,11α-dihydroxy-6β-acetoxy-6,7-*seco*-6,19-epoxy-7,20-olide-*ent*-kaur-16-en-15-one.

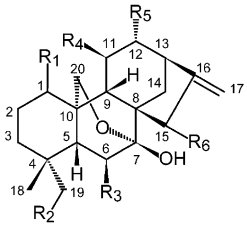
Oreskaurin B (**2**), colorless cubic crystals, was showed to have a molecular formula of $C_{22}H_{30}O_6$ by the HR-EIMS (*m*/*z* 390.2056 $[M]^+$, calcd. 390.2044). According to HMQC, ^{1}H–^{1}H COSY and HMBC experiments, the characteristic NMR signals at δ_H 3.17 and δ_H 3.21, δ_C 50.4 and δ_C 53.2 were ascribable to H-11 and H-12,



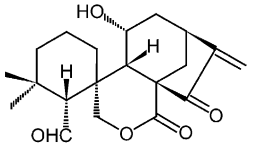
	R
1	OH
16	H



	R
2	H
15	OH



	R_1	R_2	R_3	R_4	R_5	R_6
3	H	H	OH	H	OH	β-OH
4	β-OAc	H	OAc	H	H	β-OAc
5	α-OH	H	OH	OH	H	= O
6	α-OH	OAc	OH	OH	H	= O
7	H	H	OAc	H	H	β-OH
8	β-OH	H	OH	H	H	β-OH
9	β-OH	H	OAc	H	H	β-OAc
10	α-OH	H	OH	OAc	H	β-OH



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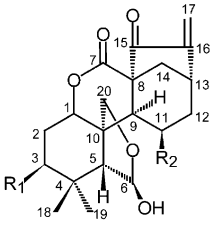
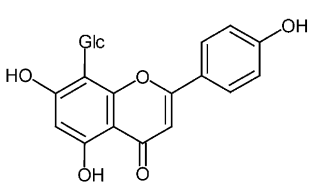


	R_1	R_2
12	H	OH
13	OH	H



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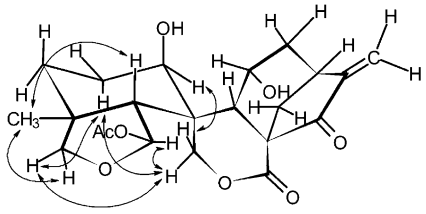

Fig. 1. Selected ROESY correlations of compound **1**.

C-11 and C-12 respectively, which implied the presence of an oxirane ring between C-11 and C-12. Comparison of the NMR spectral data of **2** with those of phyllostachysin C (**15**) (Hou et al., 2000) suggested that the only difference between compound **2** and **15** was that the hydroxyl group at C-3 of **15** was absent in **2**. The relative stereochemistry of all substituents of **2** was assigned on the basis of ROESY correlations. Thus, **2** was deduced as 7β,15β-dihydroxy-6β-acetoxy-7α,20-epoxy-11β,12β-epoxy-*ent*-kaur-16-ene.

Oreskaurin C (**3**) white amorphous powder, determined the molecular formula as $C_{20}H_{30}O_5$ according to the HR-FABMS (*m*/*z* 351.2159 $[M+H]^+$, calcd. 351.2171). The twenty carbons in **3** were characterized by ^{13}C and DEPT spectral analysis, which revealed two methyls, six methylenes (including one oxymethylene), six methines (including three oxymethines), three quaternary carbons, one ketalic carbon, and two olefinic carbons. The characteristic NMR spectra suggested that compound **3** also belongs to a diterpenoid with a 7β-hydroxy-7α,20-epoxy-*ent*-kaur-16-ene basic skeleton, which was substituted by three hydroxyl groups. A careful analysis of its 1D and 2D NMR spectra revealed that three hydroxyl groups could be placed at C-6, C-12 and C-15 respectively, as confirmed by the HMBC correlations of H-6 (δ_H 4.25, d) with C-4 (δ_C 33.9, s), C-7 (δ_C 97.4, s) and C-8 (δ_C 52.6, s); of H-12 (δ_H 4.28, m) with C-11 (δ_C 27.6, t), C-14 (δ_C 24.9, t) and C-16 (δ_C 157.5, s); and of H-15 (δ_H 5.16, s) with C-7 (δ_C 97.4, s), C-9 (δ_C 38.5, d) and C-17 (δ_C 109.2, t); this was also supported by ^1H-^1H COSY correlations of H-6 with H-5 and H-12 with H-11 (δ_H 2.03, m and 1.85, overlap). Due to the observation of the ROESY correlations between H-6α and H-19 (δ_H 1.06, s); H-12β and H-17b (δ_H 5.28, s), H-9β (δ_H 2.25, m); H-15α and H-14β (δ_H 2.27, m), OH-6, OH-12 and OH-15 were deduced as β, α and β-orientations respectively. Thus, compound **3** was established as 6β,12α,15β-trihydroxy-7α,20-epoxy-*ent*-kaur-16-ene.

Eleven known compounds were identified as enmenin monoacetate (**4**) (Mori et al., 1970), effusanin E (**5**) (Fujita et al., 1980), adenolin B (**6**) (Zhang et al, 1992), maoecrystal G (**7**) (Shen et al., 1990), enmelol (**8**) (Mori et al., 1970), trichokaurin (Enmenin) (**9**) (Mori et al., 1970), sodoponin (**10**) (Fujita et al., 1973), trichorabdal A (**11**) (Xu and Wu, 1989), nodosin (**12**) (Fujita et al., 1968), enmein (**13**) (Fujita et al., 1966), and vitexin (**14**) (Soeder and Babb, 1972) respectively, by comparing their IR, MS and NMR data with those reported in literatures. By acetylation with Ac_2O/pyridine, compound **9** was easily transformed to compound **4** (Mori et al., 1970; Fujita et al., 1969). However, this is the first time that **4** was isolated as a natural product.

Compounds **2** and **12** were tested for their ability to inhibit human tumor K562 cells, using a previously described method (Niu et al., 2002), with *cis*-platinum as a positive reference. Compound **12** showed moderate cytotoxicity with $IC_{50}=1.43$ μg/ml (*cis*-platinum: $IC_{50}=0.53$ μg/ml), while compound **2** was non-cytotoxic.

3. Experimental

3.1. General

^1H and ^{13}C NMR spectra were performed on a Bruker AM-400, and ^1H–^1H COSY, ROESY, HMQC, HMBC experiments were on DRX-500 spectrometer with pyridine-d_5 as solvent and TMS as internal standard. MS spectra were taken on a VG Auto Spec-3000 magnetic sector instrument. Optical rotations were measured on a SEPA-300 polarimeter, whereas UV spectral data were obtained using a UV-210A spectrometer. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr pellets. Column chromatography (CC) was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Factory, PR China) and D101 resin (Tianjin Agricultural Chemical Co. Ltd., P. R. China). Fractions were monitored by silica gel TLC.

3.2. Plant material

The aerial parts of *Isodon oresbius* were collected from Zhongdian County, Yunnan Province, PR China, in October 2001. The identity of plant material was verified by Prof. Xi-Wen Li, and a voucher specimen (KIB 02-01-10 Li) has been deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Chinese Academy of Sciences, PR China.

3.3. Extraction and isolation

The dried and powdered aerial parts of *I. oresbius* (1.9 kg) were extracted with 70% Me_2CO (3×2 l) at room temperature for 72 h and filtered. The filtrate was then concentrated and extracted with EtOAc (19 g) with this being applied to column chromatography over resin (D101, 200 g), eluting with MeOH–H_2O (1:1) and MeOH–H_2O (1:9) to afford two fractions A (2 g) and B (16 g). After evaporation of the solvent at reduced pressure, fraction B was applied to the silica gel (300 g) column, eluting with a gradient system of petroleum ether/acetone (1:0–0:1) to give 5 fractions B_1–B_5. Frs. B_1 and B_2 (6 g) were subjected to silica gel cc (100 g), eluting with petroleum ether/acetone (9:1–6:4) to yield rabdocoetsin D (**9**) (165 mg), enmenin monoacetate (**4**) (12 mg), maoecrystal G (**7**) (19 mg), oreskaurin B (**2**) (17 mg), trichokaurin (**9**) (21 mg) and trichorabdal A (**11**) (20 mg). Fr. B_3 (3 g) was applied to silica gel (100 g) column with petroleum ether/acetone (8:2–6:4) to yield nodosin (**12**) (23 mg), enmelol (**8**) (21 mg), sodoponin

(**10**) (25 mg) and enmein (**13**) (19 mg). Fr. B_4 (2 g) was subjected to silica gel cc (100 g) eluting with petroleum ether/acetone (7:3–5:5) yielded effusanin E (**5**) (13 mg), adenolin B (**6**) (15 mg) and oreskaurin A (**1**) (9 mg). Fr. B_5 (2 g) eluting with petroleum ether/acetone (7:3–4:6) over silica gel (100 g) column yielded oreskaurin C (**3**) (7 mg) and vitexin (**14**) (26 mg).

3.4. Oreskaurin A (*1*)

White amorphous powder: $[\alpha]_D^{20}$ −75.0° (MeOH, *c* 0.10); UV (MeOH) λ_{max} (log ϵ): 229 (3.08) nm; IR (KBr) ν_{max} cm^{-1}: 3435, 2953, 2900, 2873, 2856, 2361, 2337, 1740, 1714, 1637, 1400, 1267, 1031; FABMS *m/z* (rel. int.): 421 $[M+H]^+$ (11), 403 (6), 385 (5), 367 (6), 361 (5), 343 (6), 325 (10), 115 (100); HR-FABMS *m/z*: 421.1835 $[M+H]^+$ (calcd. for $C_{22}H_{29}O_8$, 421.1862); ^1H NMR (pyridine-d_5, 400 MHz) δ 5.96 (1H, br s, H-1β), 1.89 (1H, m, H-2β), 1.70 (1H, m, H-2α), 2.10 (1H, m, H-3β), 1.05 (1H, br d, J = 11.6 Hz, H-3α), 2.67 (1H, d, J = 3.5 Hz, H-5β), 6.41 (1H, d, J = 3.5 Hz, H-6α), 2.27 (1H, br s, H-9β), 4.48 (1H, br s, H-11β), 2.46 (1H, dd, J = 7.5, 11.9 Hz, H-12α), 1.75 (1H, br d, J = 11.9 Hz, H-12β), 3.08 (1H, dd, J = 4.0, 7.5 Hz, H-13α), 3.68 (1H, br d, J = 8.8 Hz, H-14α), 2.15 (1H, m, H-14β), 6.08 (1H, s, H-17a), 5.44 (1H, s, H-17b), 0.98 (3H, s, Me-18), 3.95 (1H, d, J = 8.0 Hz, H-19a), 3.47 (1H, d, J = 6.5 Hz, H-19b), 5.40 (1H, d, J = 10.8 Hz, H-20a), 4.73 (1H, d, J = 10.8 Hz, H-20b), 1.91 (3H, s, Me-OAc); ^{13}C NMR spectral data see Table 1.

Table 1
^{13}C NMR spectral data for compounds **1**–**3** (C_5D_5N, 100 MHz, δ in ppm)

Carbon	1	2	3
1	71.0 (d)	29.7 (t)	31.0 (t)
2	23.4 (t)	18.4 (t)	19.1 (t)
3	26.0 (t)	40.6 (t)	41.7 (t)
4	41.3 (s)	33.3 (s)	33.9 (s)
5	54.4 (d)	58.1 (d)	58.4 (d)
6	101.2 (d)	74.4 (d)	74.4 (d)
7	170.8 (s)	96.0 (s)	97.4 (s)
8	53.5 (s)	51.2 (s)	52.6 (s)
9	43.7 (d)	41.0 (d)	38.5 (d)
10	40.1 (s)	37.1 (s)	36.2 (s)
11	64.9 (d)	50.4 (d)	27.6 (t)
12	41.9 (t)	53.2 (d)	76.5 (d)
13	34.6 (d)	38.0 (d)	48.6 (d)
14	34.6 (t)	26.3 (t)	24.9 (t)
15	199.5 (s)	74.4 (d)	75.6 (d)
16	151.8 (s)	153.1 (s)	157.5 (s)
17	118.2 (t)	109.1 (t)	109.2 (t)
18	21.7 (q)	31.7 (q)	33.7 (q)
19	78.3 (t)	21.2 (q)	22.6 (q)
20	71.0 (t)	67.6 (t)	65.8 (t)
OAc	169.8 (s)	168.8 (s)	
	20.5 (q)	21.1 (q)	

3.5. Oreskaurin B (*2*)

Colorless cubic crystals: mp 167–169 °C; $[\alpha]_D^{20}$ −72.7° (MeOH, *c* 0.06); UV (MeOH) λ_{max} (log ϵ): 206 (4.22) nm; IR (KBr) ν_{max} cm^{-1}: 3428, 2935, 2868, 2361, 2337, 1750, 1653, 1646, 1450, 1220; EIMS (70 ev) *m/z* (rel. int.): 390 $[M]^+$ (1), 372 (2), 348 (1), 330 (36), 312 (11), 302 (3), 284 (11), 266 (13), 251 (7), 243 (5), 215 (7), 197 (9), 179 (10), 151 (100); HR-EIMS *m/z*: 390.2056 $[M]^+$ (calcd. for $C_{22}H_{30}O_6$, 390.2042); ^1H NMR (pyridine-d_5, 400 MHz) δ 1.78 (1H, br d, J = 11.8 Hz, H-1α) 1.28-1.50 (1H, overlap, H-1β), 1.28-1.50 (2H, overlap, H_2-2), 1.28-1.50 (1H, overlap, H-3α), 1.08 (1H, m, H-3β), 1.28-1.50 (1H, overlap, H-5β), 5.61 (1H, d, J = 3.8 Hz, H-6α), 2.59 (1H, br s, H-9β), 3.17 (1H, d, J = 3.8 Hz, H-11α), 3.21 (1H, d, J = 3.8 Hz, H-12α), 2.94 (1H, t, J = 3.8 Hz, H-13α), 2.55 (1H, br s, H-14α), 2.08 (1H, dd, J = 3.8, 12.8 Hz, H-14β), 5.01 (1H, br s, H-15), 5.55 (1H, br s, H-17a), 5.23 (1H, br s, H-17b), 0.81 (3H, s, Me-18), 1.42 (3H, s, Me -19), 4.41 (1H, d, J = 9.3 Hz, H-20a), 4.08 (1H, d, J = 9.3 Hz, H-20b), 2.16 (3H, s, Me-OAc); ^{13}C NMR spectral data see Table 1.

3.6. Oreskaurin C (*3*)

White amorphous powder: $[\alpha]_D^{20}$ −22.2° (MeOH, *c* 0.08); UV (MeOH) λ_{max} (log ϵ): 207 (3.65) nm; IR (KBr) ν_{max} cm^{-1}: 3435, 2928, 2860, 1748, 1634, 1457, 1371; EIMS (70 ev) *m/z* (rel. int.): 348 $[M-2\times H]^+$ (10), 332 (19), 314 (12), 306 (17), 288 (8), 227 (11), 209 (13), 191 (14), 151 (100); HR-FABMS *m/z*: 351.2159 $[M+H]^+$ (calcd. for $C_{20}H_{31}O_5$, 351.2171); ^1H NMR (pyridine-d_5, 400 MHz) δ 1.32 (1H, br s, H-1α), 1.19 (1H, overlap, H-1β), 1.23 (2H, overlap, H_2-2), 1.09 (2H, m, H_2-3), 1.61 (1H, d, J = 4.5 Hz, H-5β), 4.25 (1H, d, J = 4.5 Hz, H-6α), 2.25 (1H, m, H-9β), 2.03 (1H, m, H-11β), 1.85 (1H, overlap, H-11α), 4.28 (1H, m, H-12β), 3.04 (1H, d, J = 4.0 Hz, H-13α), 2.42 (1H, br d, J = 12.0 Hz, H-14α), 2.27 (1H, m, H-14β), 5.16 (1H, s, H-15α), 5.60 (1H, s, H-17a), 5.28 (1H, s, H-17b), 1.18 (3H, s, Me-18), 1.06 (3H, s, Me-19), 4.10 (2H, s, H_2-20); ^{13}C NMR spectral data see Table 1.

3.7. Cytotoxicity against K562 cell lines

The cytotoxicity assay was performed in a method of MTT, the experimental details of which have been reported previously (Niu et al., 2002).

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