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A New Diterpenoid, Taibairubescensin C, from *Isodon rubescens*

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A new kaurenoid, taibairubescensin C (**1**), was isolated from the ethanol extract of the leaves and tender branches of *Isodon rubescens* (Hemsl.) Hara. Its structure was designated as 2β,6α-diacetoxy-3β,11β-dihydroxy-*ent*-kaur-16-en-15-one (**1**) on the basis of detailed spectroscopic analysis.

Key words: *Isodon rubescens*, Labiatae, taibairubescensin C, *ent*-kaurene diterpenoid

ent-Kaurene diterpenoids with various biological activity can be isolated from plants of the genus *Isodon* (Labiatae) [1–4]. In a previous study of the chemical constituents of *Isodon rubescens* (Hemsl.) Hara, we reported on the isolation of two new *ent*-kaurene diterpenoids, taibairubescensins A and B [5]. Recently, we studied the same plant collected from Taibai Mountain to yield a new diterpenoid, taibairubescensin C (**1**), and six known diterpenoids. In this paper, we present the isolation and structure elucidation of the new diterpenoid by means of spectroscopic methods including 1D and 2D NMR.

RESULTS AND DISCUSSION

Taibairubescensin C (**1**) was obtained as an amorphous powder. The molecular formula was determined as $C_{24}H_{34}O_7$ by the positive HR-FABMS ($[M+1]^+$ *m/z* 435.2366, calcd: 435.2382) and its element analysis (found C 65.9%, H 7.4%, calcd. C 66.4%, H 7.8%). Its UV and IR spectra showed the characteristic absorption bands for a five-membered ring ketone conjugated with an *exo*-methylene (241.5 nm; 1732 and 1644 cm^{-1}). The IR spectrum also revealed hydroxyl and acetyl bands at 3396 and 1741 cm^{-1}. The ^{13}C-, DEPT-NMR (see Table 1) and ^1H-NMR spectra of **1** showed the signals for five methyl groups, four methylene groups, seven methine groups, three quaternary carbons, two olefinic carbons, a ketonic carbon and two ester carbonyl carbons. These spectral data, together with a consideration of the structure of the diterpenoids isolated from the genus *Isodon* [1], suggested that **1** possessed an *ent*-kaur-16-en-15-one skeleton with two acetoxyl groups and two hydroxyl groups (see Fig. 1).

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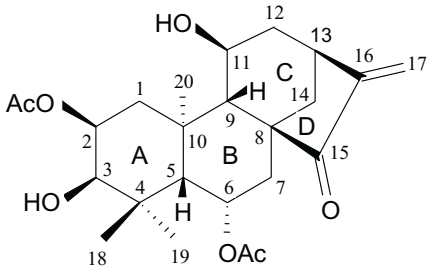

Figure 1. Structure of taibairubescensin C (**1**).

Table 1. ^{13}C-NMR data for **1** in $CDCl_3$[a].

Carbon	δ	Carbon	δ
1	39.1t	13	37.0d
2	69.9d	14	37.2t
3	77.2d	15	208.7s
4	38.8s	16	149.2s
5	46.6d	17	113.6t
6	69.0d	18	28.5q
7	41.0t	19	22.7q
8	48.6s	20	19.8q
9	62.7d	OAc	170.1s
10	39.5s		169.9s
11	65.8d		21.6q
12	37.6t		21.2q

[a] The ^{13}C NMR multiplicities were obtained by the DEPT spectrum.

The signals at δ 5.26, 5.54, 3.47 and 4.12 were assigned to the resonance of H-2, H-6, H-3 and H-11 judging from the ^{1}H-^{1}H COSY and HMBC spectra of **1** (see Table 2). In its HMBC spectrum, the signals at δ 5.26 and 5.54 exhibited correlations with the signals at δ 170.1 (s, AcO-) and 169.9 (s, AcO-), respectively, which indicated that two acetoxyl groups were attached at C-2 and C-6. Meanwhile, this result suggested that two hydroxyls were located at C-3 and C-11. The above deduction also accorded with the ^{1}H-^{1}H COSY spectrum of **1** (see Table 2).

The relative stereochemistry of **1** was established by a NOESY experiment, most of the NOESY correlations are shown in Fig. 2. The following cross-peaks were observed: from the proton signal at δ 5.26 (H-2) to the signals at δ 3.47 (H-3α) and 1.08 (H_3-19); 3.47 (H-3) to the signals at δ 1.12 (H_3-18) and 1.08 (H_3-19); 5.54 (H-6) to the signals at δ 1.76 (H-5β) and 1.58 (H-7β); and 4.12 (H-11) to the signals at δ 1.98 (H-12α), 2.22 (H-12β) and 2.64 (H-14α). On the basis of the above evidence, two acetoxyl groups at C-2 and C-6 were established as β and α-orientation, respectively;

and two hydroxyl groups at C-3 and C-11 were all assigned to be in the β-orientation. Therefore, taibairubescensin C (**1**) was elucidated as 2β,6α-diacetoxy-3β,11β-dihydroxy-*ent*-kaur-16-en-15-one.

Table 2. Principal results in ^{1}H-^{1}H COSY and HMBC spectra for **1** in $CDCl_3$[a].

Proton	^{1}H-^{1}H COSY Proton	HMBC Carbon
1α	(2α),1β	(2),3,(10)
1β	(2α),1α,20	(2)
2α	(1α),(1β),(3α)	(1),AcO(170.1ppm)
3α	(2α)	1,(2),(4),5,18,19
5β	(6β)	1,3,(4),(6),9,(10),18
6β	(5β),(7α),(7β)	8,10,AcO(169.9ppm)
7α	(6β),7β	(8),14
7β	(6β),7α	(6),(8),9,14
9β	12β,20	(8),(10),(11),15,20
11α	(12β)	8,10,(12)
12α	12β	(13),16
12β	9β,(11α),12α	(11),(13)
13α	(14β)	n.o.[b]
14α	14β	(8),(13),15,16
14β	(13α),14α	(13)
17a	17b	13,15,(16)
17b	17a	13,15
18	19	3,(4),5,19
19	18	3,(4),5,18
20	1β,9β	5,9,(10)

[a]Three-bond correlations in ^{1}H-^{1}H COSY spectrum and two-bond correlations in HMBC spectrum are indicated in parentheses.
[b]n.o. indicates no clear correlations with this proton.

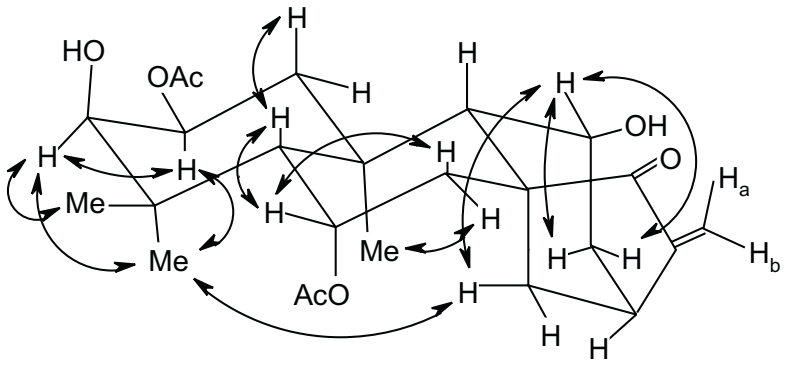

Figure 2. Mojor NOE correlations in **1**.

EXPERIMENTAL

General. IR spectrum was recorded in KBr pellets on a 170SX FT IR spectrometer. UV spectrum was obtained in MeOH on a HITACHI U-2000 spectrophotometer. Elemental analysis was measured on a PE2400 CHN elemental analyzer. Optical rotation was measured with a JASCO-20C polarimeter. HRFAB mass spectrum was recorded on an Autospec 3000 instrument. NMR spectra were recorded on a Bruker AM-400 instrument. The chemical shift values were given in ppm using TMS as the internal standard.

Plant material. The plant material of *Isodon rubescens* (Hemsl.) Hara was collected in Taibai Mountain, Shaanxi Province, P. R. China, in August 1997. A voucher specimen (SNU 97-08-01, Li) was deposited in the Herbarium of Department of Biology, Shaanxi Normal University.

Extraction and isolation. The dried powdered leaves and tender branches of *Isodon rubescens* (9.0 kg) were extracted with 95% EtOH (20000 ml×2) at room temperature for 7 days. After removal of the solvent *in vacuo*, the residue was partitioned in H_2O and extracted with petroleum ether (5000 ml×3) and EtOAc (5000 ml×3), respectively. The EtOAc extract (222.5 g) was subjected to CC on silica gel, eluting with $CHCl_3$ and increasing proportions of Me_2CO (from $CHCl_3$ 100% to Me_2CO 100%). Fractions were integrated by TLC monitoring. All components were further purified by column chromatography and preparative TLC on silica gel to give tritriacontane (854 mg, 0.0095%), taibairubescensin C (**1**, 65 mg, 0.0007%), glabcensin D (279 mg, 0.0031%), rubescensin E (526 mg, 0.0058%), rabdosianin C (112 mg, 0.0012%), trichoranin (78 mg, 0.00087%), rubescensin C (145 mg, 0.0016%), coetsoidin G (36 mg, 0.0004%), subsequently.

Taibairubescensin C (**1**) amorphous powder, $C_{24}H_{34}O_7$, elemental analysis: C 65.9%, H 7.4% (calcd. C 66.4%, H 7.8%). HR-FABMS (*m/z*): 435.2366 (calcd. 435.2382). $[\alpha]_D^{17}$ +5.0° (c 0.5, $CHCl_3$). λ_{max} (CH_3OH) nm (logε): 241.5 (3.95). IR (KBr) ν_{max} cm^{-1}: 3396, 2914, 1741, 1732, 1644, 1370, 1238, 1050. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.66 (1H, m, H-1α), 1.93 (1H, m, H-1β), 5.26 (1H, dd, J = 11.4, 4.0 Hz, H-2α), 3.47 (1H, br s, H-3α), 1.76 (1H, br s, H-5β), 5.54 (1H, br s, H-6β), 2.27 (1H, m, H-7α), 1.58 (1H, overlap, H-7β), 1.62 (1H, br s, H-9β), 4.12 (1H, d, J = 4.3 Hz, H-11α), 1.98 (1H, m, H-12α), 2.22 (1H, overlap, H-12β), 3.08 (1H, br d, J = 2.8 Hz, H-13α), 2.64 (1H, br d, J = 13.7 Hz, H-14α), 1.43 (1H, overlap, H-14β), 5.90 (1H, br s, H-17a), 5.31 (1H, br s, H -17b), 1.12 (3H, s, H-18), 1.08 (3H, s, H-19), 1.48 (3H, s, H-20), 2.08, 2.06 (each 3H, s, 2×-AcO). ^{13}C-NMR and DEPT spectra data see Table 1; the principal correlations of ^{1}H-^{1}H COSY and HMBC spectra see Table 2.

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