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ent-Kaurene diterpenoids from *Isodon rubescens*

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Abstract

Two kaurenoids, taibairubescensins A and B, were isolated from the ethanol extract of the leaves and branches of *Isodon rubescens*. Their structures are designated as 2β,3β-diacetoxy-11β,13α-dihydroxy-*ent*-kaur-16-en-15-one and 3β,11β-diacetoxy-2β,6α-dihydroxy-*ent*-kaur-16-en-15-one, respectively, on the basis of detailed spectroscopic analyses.

Keywords: *Isodon rubescens*; Labiatae; Taibairubescensins A and B; *ent*-Kaurene diterpeniods

1. Introduction

Plants of genus *Isodon* (Labiatae) have proven to be a rich source of *ent*-kaurene diterpenoids with varied biological activity (Fujita & Node, 1984; Cheng, Guo & Xu, 1987; Zhao et al., 1989; Zhao, Tian, Yue, Lin & Sun, 1997). Previous studies on *Isodon rubescens* led to the isolation of more than 10 diterpenoids (Sun, Pan, Lin & Niu, 1988). In further study of the minor diterpenoid constituents of this species, we have isolated two new diterpenoids, taibairubescensins A (**1**) and B (**2**), one known diterpenoid, rabdoforrestin A, and two other known compounds, α-amyrin and ursolic acid. In this paper, we present the isolation and structure elucidation of the new diterpenoids by means of spectroscopic methods including 1D- and 2D-NMR.

2. Results and discussion

Taibairubescensin A (**1**) was obtained as an amorphous powder. The molecular formula was determined as $C_{24}H_{34}O_7$ by FABMS ([M + 1] *m*/*z* 435). Its UV and IR spectra showed characteristic absorption bands for a five-membered ring ketone conjugated with an *exo*-methylene (240.5 nm; 1732 and 1649 cm^{-1}). In addition, the IR spectrum also revealed hydroxyl and acetyl bands at 3468 and 1740 cm^{-1}. The ^1H-, ^{13}C- and DEPT-NMR spectra of **1** showed signals for five methyl groups, five methylene groups, five methine groups, four quaternary carbons, two olefinic carbons, a ketonic carbon and two ester carbonyl carbons. Considering the structures of diterpenoids from the genus *Isodon*, these data suggested that **1** possessed an *ent*-kaur-16-en-15-one skeleton with two acetoxyls and two hydroxyls.

The ^{13}C-NMR data of **1** were very similar to those of deacetylisodopharicin A (**3**) (Wang et al., 1991) except for one more acetyl groups. Comparison of their ^{13}C-NMR data indicated that the only difference between **1** and **3** was in the A ring i.e. two acetoxyls were located on the A ring of compound **1**. In the ^1H–^1H COSY spectrum of **1**, signal at δ 4.98 (1H, *d*, J = 2.6 Hz, H-3α) showed a cross peak with the signal at δ 5.24 (1H, *ddd*, J = 12.3, 2.6, 3.9 Hz, H-2α), which exhibited cross peaks with signals at δ 1.59 (1H, *dd*, J = 11.9, 3.9 Hz, H-1α) and δ 1.91 (1H, *dd*, J = 11.9, 12.3 Hz, H-1β). In the HMBC spectrum of **1**, signals

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at δ 1.59 (H-1α) and δ 1.91 (H-1β) correlated with signals at δ 39.6 ppm (C-10) and δ 67.5 ppm (C-2), whereas the latter showed a correlation with the signal at δ 4.98 (H-3α), which correlated with the signals at δ 38.1 ppm (C-4) and δ 170.4 ppm (OAc). The signal at δ 76.6 ppm (C-3) correlated with the signal at δ 5.24 (H-2α), which exhibited a cross peak with the signal at δ 37.9 ppm (C-1). Thus, two acetoxyl groups were assigned at C-2 and C-3, respectively. Moreover, the signal at δ 75.3 ppm (C-13) correlated with the signals at δ 5.98 (1H, *br s*, H-17a), δ 5.41 (1H, *br s* H-17b) and δ 2.05 (1H, *dd*, J = 13.9, 4.8 Hz, H-12α), δ 2.30 (1H, *dd*, J = 13.9, 4.8 Hz, H-12β). Both signals at δ 2.05 and δ 2.30 exhibited correlation with the signal at δ 66.7 ppm (C-11), which showed a cross peak with the signal at δ 1.51 (1H, *br s*, H-9β). These results

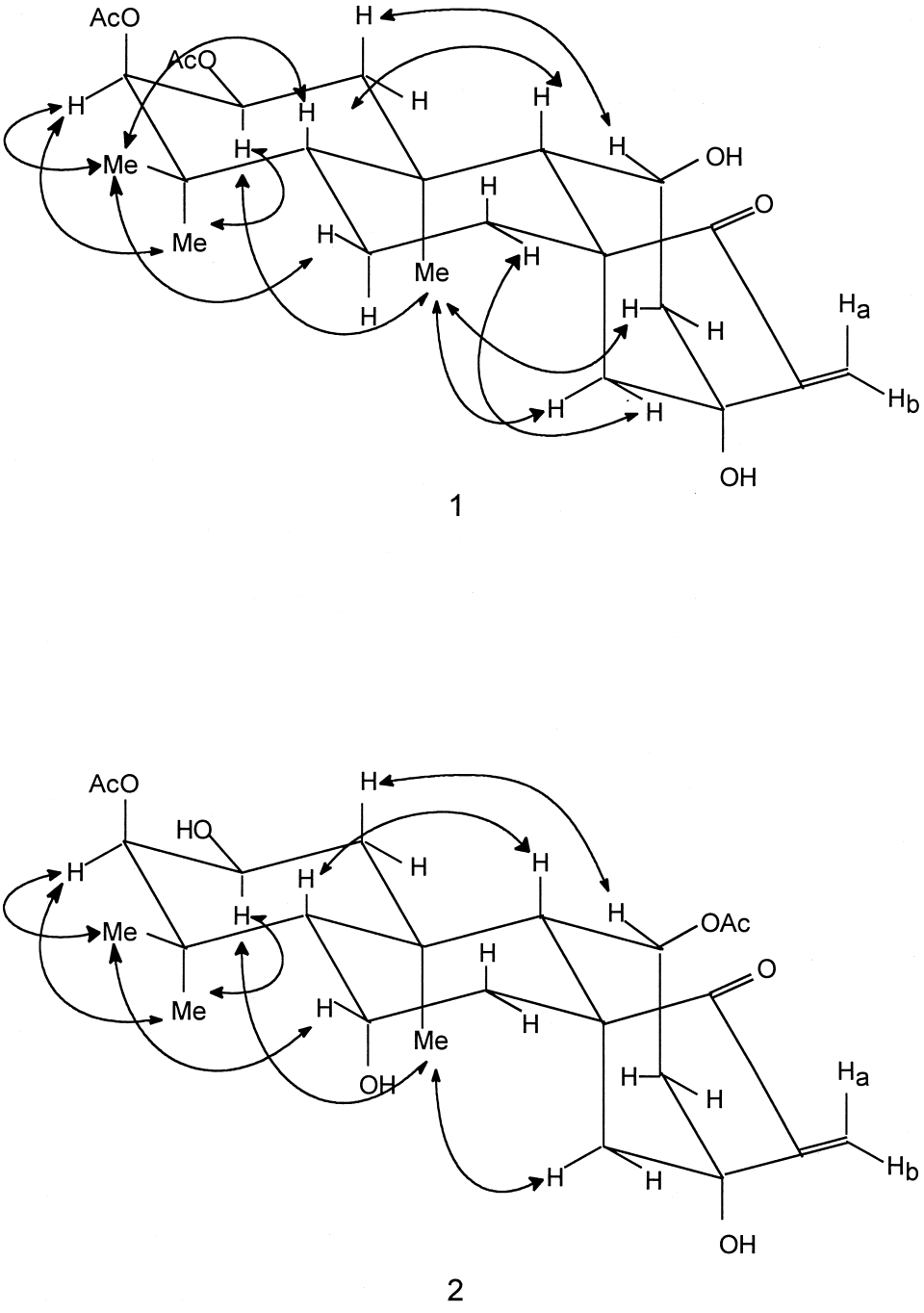


Fig. 1. Major NOE correlations in **1** and **2**.

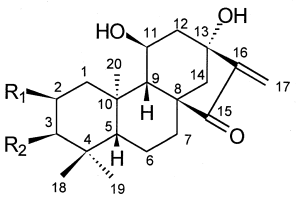
suggested that two hydroxyl groups were located at the C-11 and C-13 positions.

The relative configurations of the 2-OAc and 3-OAc were established as 2β-OAc and 3β-OAc by considering the coupling constants of H-2α with H-1α and H-1β, and H-2α with H-3α, respectively. The hydroxyl group at C-11 of **1** should be in the β-orientation on the basis of the coupling constants of H-11α with H-12α and H-12β. The hydroxyl group at C-13 position is in α-orientation judging from the fact that H-13 is in the α-orientation in typical *ent*-kaurene diterpenoids which are unsubstituted at C-13. The unambiguous establishment of the stereochemistry of **1** was confirmed by a NOESY experiment; most of the NOESY correlations are shown in Fig. 1. Therefore, **1** was elucidated as 2β,3β-diacetoxy-11β,13α-dihydroxy-*ent*-kaur-16-en-15-one.

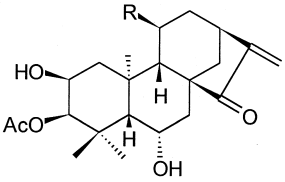
Taibairubescensin B (**2**) was obtained as an amorphous powder. The positive HR-FABMS indicated a molecular formula of $C_{24}H_{34}O_7$ (435.2364, calc. 435.2382). Its UV and IR spectra showed characteristic absorption bands for a five-membered ring ketone conjugated with an *exo*-methylene at 243.5 nm; 1738 and 1648 cm^{-1}, respectively. The IR absorption band at 3473 cm^{-1} revealed the presence of hydroxyl groups. The ^{1}H-, ^{13}C- and DEPT-NMR spectra of **2** clearly indicated that it is a typical *ent*-kaurene diterpenoid derivative with two acetoxyls and two hydroxyls.

The ^{1}H-NMR spectroscopic data of **2** were very similar to those of lusanrubescensin D (**4**) (Qin, Li, Li, Sun & Lin, 1986) except for the presence of one more acetyl group. The ^{13}C-NMR data of **2** differed from those of **4** (Qin et al., 1986) only at C-11. The downfield shift of C-11 from δ 65.1 ppm in **4** to δ 68.5 ppm in **2** indicated that the acetoxyl group at the C-11 in **2** had replaced a hydroxyl group in **4**. Therefore, two hydroxyl groups were located at C-2 and C-6 just as in **4**, and two acetoxyl groups were located at C-3 and C-11 in **2**. These assignments were further confirmed by the HMBC spectrum of **2** (see Table 1). Signals at δ 4.86 (1H, *d*, J = 3.3 Hz, H-3α) and δ 5.50 (1H, *br s*, J = 1.2 Hz, H-11α) exhibited cross peaks with signals at δ 171.9 ppm and δ 169.8 ppm, respectively. Thus, two acetoxyl groups were assigned to C-3 and C-11, and two hydroxyl groups were assigned to C-2 and C-6.

The relative configurations of the substituents of **2** were established through analysis of the coupling constants and NOESY spectrum; most of the NOESY correlations are shown in Fig. 1. On the basis of the above evidence, the structure of **2** was determined to be 3β,11β-diacetoxy-2β,6α-dihydroxy-*ent*-kaur-16-en-15-one.



1. R_1 = OAc, R_2 = OAc
3. R_1 = H, R_2 = OH



2. R = OAc
4. R = OH

Table 1
Principal HMBC correlations of **1** and **2**[a]

Proton	**1**	**2**
1α	(2), 3, 9, (10)	(2), 3, 5, 9, (10)
1β	(2), 3, 9, 20, (10)	(2), 3, 5, (10)
2α	(3)	n.o.[b]
3α	(2), (4), 5, 19, OAc (170.4 ppm)	1, (2), (4), 5, 19, OAc (171.9 ppm)
5β	(4), (6), 7, (10), 20	3, (4), 18, 19, 20
6α	(5), (7)	–
6β	(5), (7)	4, (5)
7α	(6), (8), 14	5, 14
7β	5, (6), (8), 9	5, (6), 14
9β	(8), (10), (11), 20	(8), (11), 12, 20
11α	8, 10, 13	8, 10, OAc (169.8 ppm)
12α	9, (11), (13), 14	9, (11), (13)
12β	(13), 14	n.o.
13α	–	n.o.
14α	(8), 9, 12, (13), 15, 16	(8), (13), 15, 16
14β	(8), 9, 12, (13), 15,	(8), (13), 15, 16
17a	13, 15, (16)	13, 15, (16)
17b	13, 15, (16)	13, 15
18	3, (4), 5, 19	3, (4), 5, 19
19	3, (4), 5, 18	3, (4), 5, 18
20	1, 5, 9, (10)	1, 5, 9, (10)

[a] Two-bond correlations are indicated in parentheses.
[b] n.o. indicates no clear correlations with this proton.

3. Experimental

3.1. General

IR spectra were recorded in KBr pellets on a 170SX FT IR spectrometer. UV spectra were recorded in MeOH on a HITACHI U-2000 spectrophotometer. The optical rotations were measured with a JASCO-20C polarimeter. EI mass spectrum was recorded on a HP 5988A instrument. FAB and HR mass spectra were recorded on an Autospec 3000 instrument. NMR spectra were recorded on a Bruker AM-400 instrument. The chemical shift values are given in ppm using TMS as the internal standard.

3.2. Plant material

The plant material of *I. rubescens* 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 the Department of Biology, Shaanxi Normal University.

3.3. Extraction and isolation

The dried powdered leaves and branches of *I. rubescens* (9.0 kg) were extracted with 95% EtOH (20,000 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). The EtOAc extract (222.5 g) was subjected to CC on silica gel, eluting with $CHCl_3$ and increasing proportions of Me_2CO. Fractions were combined by monitoring with TLC. All components were further purified by column chromatography and preparative TLC on silica gel to give taibairubescensin A (**1**, 472 mg, 0.005%), taibairubescensin B (**2**, 390 mg, 0.004%), rabdoforrestin A (4.6 g, 0.05%), α-amyrin (240 mg, 0.003%) and ursolic acid (1.9g, 0.02%).

3.4. Taibairubescensin A (*1*)

Amorphous powder; $[\alpha]_D^{17}$ 49.3° (*c* 0.5, $CHCl_3$); λ_{max}^{MeOH} nm (log): 240.5 (3.91); IR ν_{max}^{KBr} cm^{-1}: 3468, 2943, 1740, 1732, 1649, 1257, 1234, 1039 and 980; FABMS *m/z*: 435 [M + 1]; ^{1}H-NMR spectral data (400 MHz, $CDCl_3$): 1.59 (1H, *dd*, *J* = 11.9, 3.9 Hz, H-1α), 1.91 (1H, *dd*, *J* = 11.9, 12.3, Hz, H-1β), 5.24 (1H, *ddd*, *J* = 12.3, 2.6, 3.9 Hz, H-2α), 4.98 (1H, *d*, *J* = 2.6 Hz, H-3α), 1.43 (1H, *br s*, H-5β), 1.36 (1H, *m*, H-6α), 1.66 (1H, *m*, H-6β), 2.14 (1H, *m*, H-7α), 1.44 (1H, *m*, H-7β), 1.51 (1H, *br s*, H-9β), 4.20 (1H, *t*, *J* = 4.8 Hz, H-11α), 2.05 (1H,*dd*, *J* = 13.9, 4.8 Hz, H-12α), 2.30 (1H, *dd*, *J* = 13.9, 4.8 Hz, H-12β), 2.50 (1H, *br d*, *J* = 11.3 Hz, H-14α), 1.47 (1H, *d*, *J* = 11.3 Hz, H-14β), 5.98 (1H, *br s*, H-17a), 5.41 (1H, *br s*, H-17b), 0.90 (3H, *s*, Me-18), 0.99 (3H, *s*, Me-19), 1.13 (3H, *s*, Me-20), 1.97, 2.12 (each 3H, *s*, OAc × 2); ^{13}C-NMR spectral data are listed in Table 2.

3.5. Taibairubescensin B (*2*)

Amorphous powder; $[\alpha]_D^{17}$ −34.0° (*c* 1, $CHCl_3$); λ_{max}^{MeOH} nm (log): 243.5 (3.82); IR ν_{max}^{KBr} cm^{-1}: 3474, 2935, 1738, 1648, 1375, 1242, 1048 and 981; HR-FABMS *m/z*: 435.2364 $[M + 1]^+$ for $C_{24}H_{34}O_7$ (calc. 435.2382); EIMS (70 eV) *m/z* (rel. int.): 434 (1), 390 (1), 374 (1), 314 (1), 299(1), 281 (3), 263 (1), 257 (7), 235 (1), 197 (1), 150 (3), 105 (5), 43 (100); ^{1}H-NMR spectral data (400 MHz,$CDCl_3$): 1.37 (1H, *br d*, *J* = 12.2 Hz, H-1α), 1.95 (1H, *dd*, *J* = 12.2, 4.0 Hz, H-1β), 4.19 (1H, *ddd*, *J* = 11.9, 4.0, 3.3 Hz, H-2α), 4.86 (1H, *d*, *J* = 3.3 Hz, H-3α), 1.54(1H, *br s*, H-5β), 4.14 (1H, *br d*, *J* = 4.4 Hz, H-6β), 2.09 (1H, *dd*, *J* = 7.3, 4.4 Hz, H-7α), 2.01 (1H, *br d*, *J* = 7.3 Hz, H-7β), 1.61 (1H, *br s*, H-9β), 5.50 (1H, *br d*, *J* = 1.2 Hz, H-11α), 1.45 (1H, *m*, H-12α), 1.59 (1H, *m*, H-12β), 3.08 (1H, *br d*, *J* = 3.4 Hz, H-13α), 2.61 (1H, *dd*, *J* = 13.1, 5.6 Hz, H-14α), 2.23 (1H, *dd*, *J* = 13.1, 3.4 Hz, H-14β), 5.88 (1H, *br s*, H-17a), 5.31 (1H, *br s*, H-17b), 0.98 (3H, *s*, Me-18), 1.10 (3H, *s*, Me-19), 1.43 (3H, *s*, Me-20), 2.14, 2.04 (each 3H, *s*, OAc × 2); ^{13}C-NMR spectral data; see Table 2.

Table 2
^{13}C-NMR spectral data for **1** and **2** in $CDCl_3$

Carbon	**1**	**2**
1	37.9	43.2
2	67.5	65.0
3	76.6	80.9
4	38.1	38.4
5	48.6	48.4
6	17.6	65.8
7	32.5	41.1
8	52.8	48.4
9	61.9	62.9
10	39.6	39.4
11	66.7	68.5
12	48.2	37.3
13	75.3	37.0
14	44.9	37.7
15	207.3	208.7
16	151.9	149.1
17	113.7	113.7
18	27.8	28.2
19	21.3	22.6
20	18.3	19.9
OAc	170.4	171.9
	170.6	169.8
	20.9	21.1
	21.0	21.6

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