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Tetrahedron
Tetrahedron 60 (2004) 2373–2377

Two novel tricyclic diterpenoids from *Isodon rubescens* var. *taihangensis*

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Received 6 August 2003; revised 27 October 2003; accepted 6 January 2004

Abstract—Two novel tricyclic diterpenoids rubescensins U (**1**) and V (**2**) were isolated from the leaves of *Isodon rubescens* var. *taihangensis*. They were elucidated as a 8,15-*seco*-*ent*-kauranoid and an *ent*-abietanoid, respectively, by 1D and 2D NMR spectra, and single crystal X-ray analysis. Compound **1** is the first example of an 8,15-*seco*-*ent*-kaurane from the plants genus *Isodon*. A discussion of their biogenesis is described.

1. Introduction

In recent years, a series of tricyclic diterpenoids were reported from the genus *Isodon*, which was well-known to be abundant in tetracyclic *ent*-kaurane diterpenoids.[1] Among them, adenanthin L (**3**) from *I. adenantha*,[2] laxiflorin O (**4**) from *I. eriocalyx* var. *laxioflora*,[3] and eriocaside A (**5**) from *I. eriocalyx*,[4] were elucidated as *ent*-abietanoids; Melissoidesin L (**6**) from *I. Melissoides*,[5] was an abietanoid, and taibaihenryiin C (**7**) from *I. henryi* was even regarded as having a novel skeleton,[6] on the basis of their tricyclic skeleton. In our continuing research for more bioactive substances from the *Isodon* plants, two tricyclic diterpenoids (**1** and **2**) were isolated from *Isodon rubescens* var. *taihangensis* Z. Y. Gao and Y. R. Li,[7] a famous folk herbal medicine for treatment of cancers.[8] Compounds **1** and **2** were determined as a 8,15-*seco*-*ent*-kauranoid and an *ent*-abietanoid by the key H-8β of **1** and H-8α of **2**, respectively. From the similarity in the structures of compounds **1** and **2**, a brief discussion of their biogenesis is described.

2. Results and discussion

Compound **1** was obtained as colorless, prismatic crystals with a molecular formula $C_{20}H_{28}O_6$ determined by the HREIMS. The 20 carbon atoms found in the ^{13}C and DEPT NMR spectra of **1** consisted of a ketonic carbon, an aldehydic carbon, an olefinic quaternary carbon, an olefinic methylene carbon, a hemiacetal carbon, seven methine carbons including three oxygenated ones, four methylene carbons, two quaternary carbons, and two methyl carbons, which obviously suggested a diterpene skeleton. Compound **1** was further deduced to be a tricyclic diterpenoid by the absence of a quaternary carbon found in other typical *ent*-kauranoids also isolated from the same plant, such as lasiodonin (**8**),[9] and the presence of H-8 clearly exhibiting HMBC correlations with C-10, C-11, and C-13 (Table 1). Because H-8 has been determined to be of a β orientation by the ROESY correlations of H-8/H-5β and H-8/H-9β, and considering the structures of diterpenoids isolated from this plant, compound **1** was deduced to be a 8,15-*seco*-*ent*-kauranoid, instead of an *ent*-abietanoid.

The remaining oxygenated functionalities of **1** were established accordingly. OH-1α and OH-7α were deduced by the HMBC correlations of H-1/C-5 and C-9, H-7/C-5 and C-9 (Table 1), and the ROESY correlations of H-1β/H-5β and H-7β/H-8β (Fig. 1). The ketonic carbon was assigned as C-6 by the long-range correlations of H-5 and H-7 with C-6 in the HMBC spectrum. Based on the analysis of the relational HMBC correlations of **1** (Table 1), the olefinic bond conjugated with the aldehydic group was located at C-13. The 11,20-epoxy group was also deduced in the same way. Consequently, with the aid of the NOEs of H-11α/H-13α and H-20/Me-19 in the ROESY spectrum, compound **1**

Keywords: Labiatae; *Isodon rubescens* var. *taihangensis*; 8,15-*seco*-*ent*-Kauranoid; *ent*-Abietanoid; Rubescensin U; Rubescensin V.
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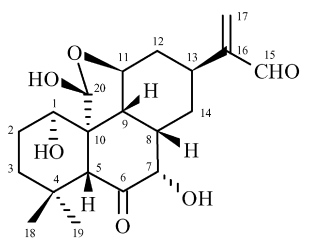
doi:10.1016/j.tet.2004.01.028

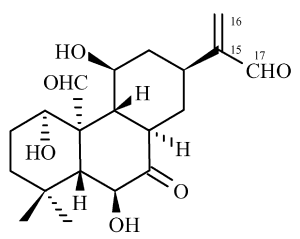
Table 1. NMR spectral data and HMBC correlations for **1** and **2**[a]

No.	**1**			**2**		
	^{1}H	^{13}C	HMBC[b]	^{1}H	^{13}C	HMBC[b]
1	3.92–3.95 m	76.9 d	9, 20	4.22–4.27 m	74.7 d	2, 3, 5, 9, 20
1-OH	6.15 d, 8.0		1, 10	7.95 s		
2	2.80–2.84 m	29.8 t	4, 10	2.10–2.15 m	28.9 t	1, 3, 4, 10
	2.00–2.06 m			2.03–2.08 m		
3	1.51 overlap	41.4 t	1, 4, 5, 18, 19	1.60–1.65 m	39.7 t	1, 2, 4, 5
	1.38 dt, 4.0, 13.2			1.39–1.45 m		
4		32.7 s			34.9 s	
5	2.49 s	57.8 d	6, 7, 9, 18, 19, 20	1.66 d, 10.0	57.5 d	6, 7, 9, 10, 18, 19
6		214.8 s		4.67 d, 10.0	75.2 d	4, 5, 7
6-OH				8.02 s		
7	4.52 d, 8.0	77.7 d	5, 6, 8, 9		210.8 s	
7-OH	6.88 s		6, 8			
8	3.08–3.11 m	37.2 s	10, 11, 13	3.37 dt, 2.2, 12.0	47.5 d	7, 9, 11, 14
9	2.68 dd, 3.6, 11.0	52.6 d	1, 5, 7, 8, 12	1.71 br t, 12.0	59.9 d	5, 8, 10, 11, 20
10		54.4 s			58.7 s	
11	3.66 dt, 3.0, 11.0	72.5 d	8, 10, 20	4.40–1.45 m	70.3 d	8, 9, 12
11-OH				5.79 br s		
12	2.32 overlap	36.7 t	9, 13, 14, 16	2.36–2.41 m	41.9 t	9, 11, 13, 14, 16
	1.58–1.63 m			1.51–1.55 m		
13	3.81–3.85 m	33.4 d	8, 11, 14, 15, 17	2.72 br t, 12.5	32.8 d	16
14	2.28 overlap	31.9 t	8, 9, 13, 16	2.25 dd, 2.2, 13.2	31.8 t	7, 8, 9, 12, 16
	1.41–1.46 m			1.45–1.49 m		
15	9.53 s	194.5 d	13, 17		153.6 s	
16		154.8 s		6.19, 5.89 (each 1H, s)	133.4 t	13, 15, 17
17	6.12, 5.84 (each 1H, s) s	133.6 t	13, 15	9.55 s	194.4 d	13, 16
18	1.01 s (3H)	30.3 q	3, 4, 5, 19	1.40 s (3H)	34.0 q	3, 4, 5, 19
19	1.67 s (3H)	21.0 q	3, 4, 5, 18	1.27 s (3H)	23.1 q	3, 4, 5, 18
20	5.73 d, 8.0	103.2 d	1, 5, 9, 11	10.73 s	207.9 d	10
20-OH	8.47 d, 8.0		10, 20			

[a] ^{1}H NMR, 400 MHz; ^{13}C NMR, 100 MHz, pyridine-d_5; data in ppm (J in Hz).
[b] From H to C.



1



2

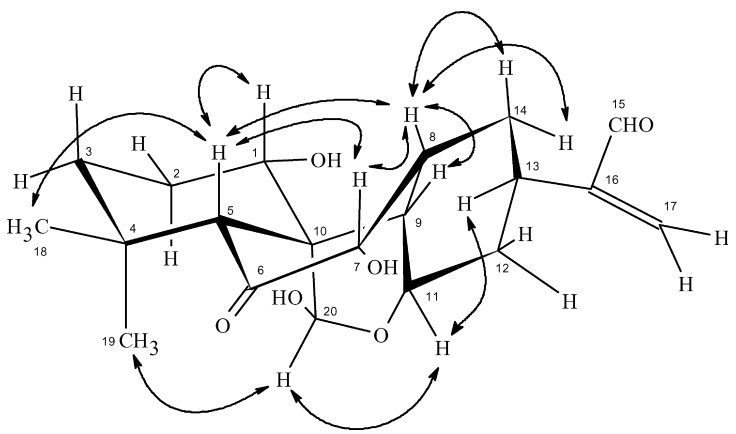


Figure 1. Selected ROESY correlations for **1**.

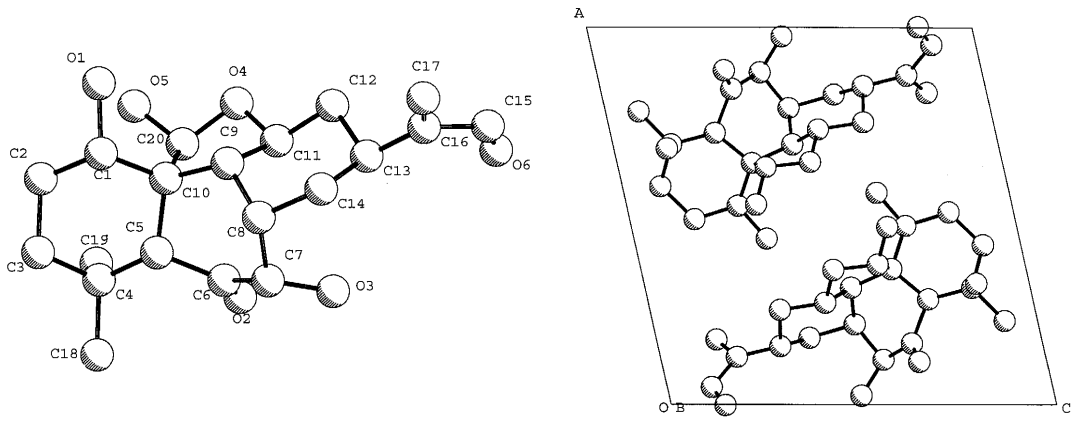


Figure 2. Crystal structure of **1**.

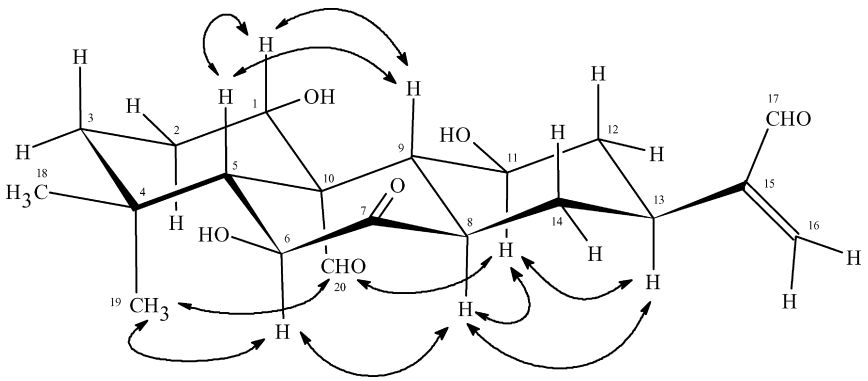


Figure 3. Selected ROESY correlations for **2**.

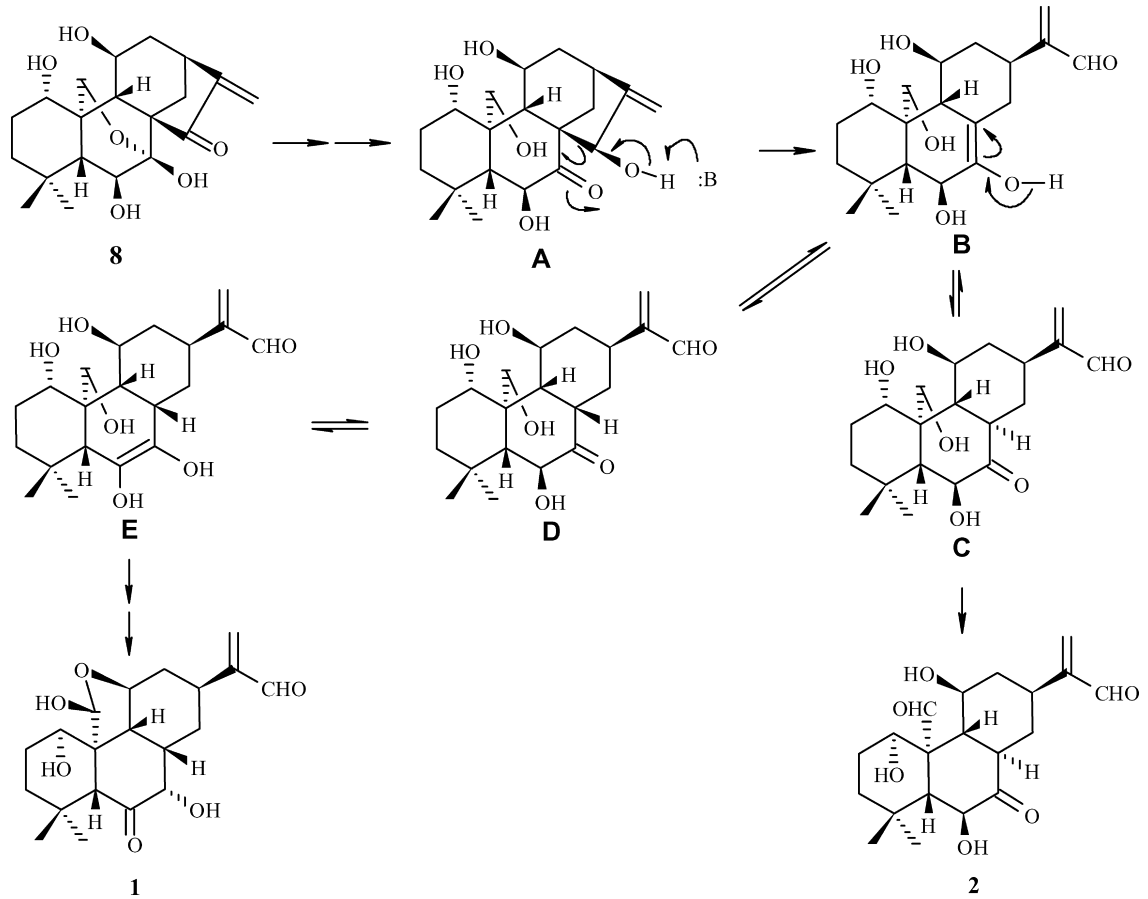


Figure 4. A plausible biogenetic pathway to account for the formation of compounds **1** and **2**.

was elucidated as 20(*S*)-1α,7α,20-trihydroxy-6,15-dioxo-11β,20-epoxy-8,15-*seco*-*ent*-kaur-16(17)-ene, named rubescensin U (**1**). Finally, the X-ray crystallographic analysis of **1** (Fig. 2) confirmed stereochemically that rings A and C were in chair forms, and ring B showed a twist boat conformation. This 8,15-*seco*-*ent*-kaurene skeleton was proved and reported from the genus *Isodon* plants for the first time.[10]

Similarly, compound **2** was also educed to be a tricyclic diterpenoid. A comparison of the ^{1}H and ^{13}C NMR data of compounds **2** and **1** suggested that **2** was derived from the hydrolysis of the hemiacetal group at C-20 of **1**. Further analysis of the ROESY spectrum of **2** revealed a key difference between **2** and **1**, in that the H-8 of **2** showed NOEs with H-13α and H-11α (Fig. 3) instead of correlating with H-5β and H-9β, indicating the α-orientation of H-8. This presence of H-8α was confirmed by the coupling constant (J=12.0 Hz) between H-8 and H-9β, and indicated that **2** is an *ent*-abietanoid. Accordingly, by the ROESY correlation of H-6α/Me-19, compound **2** was established as 1α,6β,11β-trihydroxy-7,17,20-trioxo-*ent*-abiata-15(16)-ene, and named rubescensin V.

The biogenesis from lasiodonin **8**, one of the major *ent*-kauranoids of this plant, to compounds **1** and **2** was postulated (Fig. 4) to explain their origins. In the proposed biogenetic pathway, a retroaldol reaction from **A** to **B** resulted in the key transformation that converted a tetracyclic *ent*-kaurane to a tricylic diterpenoid.[11,12] The keto-end equilibration of **B** gave **C** and **D**, and determined the key stereochemical difference between **1** and **2**. The subsequent enolization from **D** to **E**, oxidation and hemiacetalization yielded **1**. Compound **2** was derived from the oxidation of **C**. Thus, the *ent*-abietanoid **2** could have originated from an *ent*-kaurane.

3. Experimental

3.1. General procedures

Melting points were measured on an XRC-1 micro melting point apparatus and were uncorrected. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. IR spectra were obtained on a Bio-Rad FtS-135 spectrophotometer with KBr pellets. MS were recorded on a VG Auto Spec-3000 spectrometer. 1D- and 2D NMR spectra were obtained on the Bruker AM-400 and DRX-500 instruments with TMS as an internal standard.

3.2. Plant material

The leaves of *Isodon rubescens* var. *taihangensis* were collected from Hebi Prefecture, Henan Province, in August 2000, and identified by Professor Z. W. Lin, Kunming Institute of Botany. A voucher specimen has been deposited in the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

3.3. Extraction and isolation

The 70% Me_2CO extracts of the air-dried and powdered leaves of *I. rubescens* var. *taihangensis* (10 kg) were partitioned with EtOAc to afford the EtOAc extract (400 g), which was subjected to silica gel column chromatography using $CHCl_3$, $CHCl_3$–Me_2CO (9:1, 8:2, 7:3, 6:4) and Me_2CO as eluents. Compounds **1** and **2** (14 and 6 mg) were obtained from the $CHCl_3$–Me_2CO (7:3) fraction after repeated silica gel column chromatographic separations, followed by preparative TLC and recrystallization from MeOH.

3.3.1. Compound 1. Colorless prismatic crystals. Mp 202–204 °C; $[\alpha]_D^{21.6}=-60.0$ (c=0.1, acetone); IR (KBr) ν_{max}: 3433, 2928, 1716, 1683, 1683, 1124 cm^{-1}. ^{1}H NMR (C_5D_5N, 400 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz): see Table 1; EI-MS (70 eV) m/z (%): 364 (M^+, 3), 346 (20), 328 (8), 318 (40), 300 (15); HREIMS m/z: $[M]^+$ 364.1897 (calcd for $C_{20}H_{28}O_6$ 364.1886).

Crystal data for **1**. Crystals of **1**, crystallized from methanol, belong to the monoclinic space group $P2_1$. Crystal data: $C_{20}H_{28}O_6{\cdot}H_2O$, M=364.43, a=12.368(2), b=6.275(1), c=12.289(2) Å, β=102.76(1)°, V=930.2(3) $Å^3$, Z=2, d=1.301 g/cm^{-3}, Mo Kα radiation, linear absorption coefficient μ=1.0 cm^{-1}. A colorless quadrate lumpish crystal of dimensions 0.02×0.15×0.60 mm^3 was used for X-ray measurements on a MAC DIP-2030 diffractometer with a graphite monochromator, maximum 2θ value of 50.0° was set. The total number of independent reflections measured was 1530, 1431 of which were considered to be observed ($|F|^2 \geq 8\sigma|F|^2$). The structure was solved by the direct method SHELX-86 and expanded using difference Fourier techniques, refined by the program and method NOMCSDP[13] and full-matrix least-squares calculations. Hydrogen atoms were fixed at calculated positions. The final indices were R_f=0.071, R_w=0.070 ($w=1/\sigma|F|^2$).

3.3.2. Compound 2. White amorphous powder; $[\alpha]_D^{21.4}=-5.0$ (c=0.2, acetone); IR (KBr) ν_{max}: 3441, 2928, 1705, 1683, 1084 cm^{-1}. ^{1}H NMR (C_5D_5N, 400 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz): see Table 1; (+) FAB-MS m/z: 365 ($[M+1]^+$); (+) HRFABMS m/z: $[M+H]^+$ 365.1987 (calcd for $C_{20}H_{29}O_6$ 364.1964).

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