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Diterpenoids from *Isodon parvifolius*

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Abstract: A new *ent*-kaurane diterpenoid, 2*β*,3*β*,6*β*,11*β*-tetraacetoxy-*ent*-kaur-16*β*-methy-15-one (**1**), along with three kown diterpenoids, hebeirubescensin L (**2**), rabescensin C (**3**), and trichokaurin (**4**) were isolated from the leaves of *Isodon parvifolius*. Their structures were elucidated on the basis of spectroscopic methods and literatures.

Keywords: *Isodon parvifolius*; *ent*-kaurane diterpenoid.

1. Introduction

The genus *Isodon* (Labiatae family) have attracted considerable attention being a rich source of *ent*-kaurane diterpenoids, which have diverse structures and bioactivities [1–2]. *Isodon parvifolius* (Batalin) H. Hara is mainly distributed in Sichuan Province, Shanxi Province, and the Tibet Autonomous Region, People's Republic of China. Various types diterpenoids have been found from this plant, including *ent*-isopimarane, *ent*-abietanoids, *ent*-labdane, and *ent*-kaurane type diterpenoids [3–6]. In search for new natural substances, a further study of this species collected from Sanxi Province led to the isolation of a new *ent*-kauranoid parvifonin (**1**), along with three known diterpenoids hebeirubescensin L (**2**), rabescensin C (**3**), and trichokaurin (**4**) (Figure 1). In this paper, we describe the isolation and structure elucidation of compound **1**.

2. Materials and Methods

2.1. General

Optical rotation was carried out on a Perkin-Elmer model 241 polarimeter. UV spectra was obtained using a Shimadzu UV-2401A. IR spectra wase recorded as KBr pellets on a Perkin-Elmer 599B spectrophotometer. MS was determined on a Bruker Daltonics Apex III mass spectrometer. NMR spectra were measured on Bruker DRX-500 spectrometers with TMS as int. standard and $CDCl_3$ as solvent. Silica gel (80–100 and 200–300 mesh，Qingdao Marine Chemical Co., China), MCI

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(CHP20/P50, MITSUBISHI) and reversed-phase C-18 silica gel (60 mesh, Merck) were used for column chromatography. The silica gel GF254 for TLC (Qingdao Marine Chemical Co., China). Agilent 1220LC equipped with an Welchrom C-18 column (4.6 × 250 mm) was used for HPLC analysis and a semi-preparative Welchrom C-18 column (10 × 250 mm) was used in sample preparation.

2.2. Plant Material

The aerial parts of *I. parvifolius* were collected in the Qinling Mountain, Shanxi Province, People's Republic of China, in July 2008, and identified by Prof. Yonghui Feng, Xi'an Medical University. A voucher specimen (No. Xiyi-2008010) was deposited in the Herbarium of Xi'an Medical University, China.

2.3. Extraction and isolation

Air-dried and powdered leaves of *I. parvifolius* (1.2 kg) were extracted by 75% ethanol three times (each 3 day and 5 L) at room temperature. The extracts were condensed in vacuo to afford crude residue (220 g) which was suspended in water (600 mL) and subsequently partitioned successively with EtOAc to afford EtOAc fraction. The EtOAc fraction (75 g) was decolorized on MCI gel, eluted with 90% MeOH–H_2O to yield a pale yellow gum (55 g). The gum was subjected to column chromatography on silica gel using a step gradient elution of $CHCl_3$–Me_2CO (1:0–0:1). The fractions obtained from $CHCl_3$–Me_2CO (1:1) elution were combined and subjected to repeated column chromatography and further purified by preparative HPLC to yield pure compounds **1** (5 mg), **2** (8 mg), **3** (11mg), and **4** (5 mg) .

Compound **1**, 2*β*,3*β*,6*β*,11*β*-tetraacetoxy-*ent*-kaur-16*β*-methy-15-one, named parvifonin, white amorphous powder, $C_{28}H_{40}O_9$ by HRESIMS, $[\alpha]^{19}_{D}$. – 23.08° (C 0.84, CH_3OH); UV (CH_3OH): λ_{max} (log ε) 204 (10.2) nm; IRν_{max} (KBr. cm^{-1}): 3424, 1738, 1628, 1244; HRESIMS $[M + Na]^+$ *m*/*z* 543.2579 (calcd. for 543.2570) for $C_{28}H_{40}O_9Na$); ^1H and ^{13}C NMR data see Table.

Table. ^1H and ^{13}C NMR data (in $CDCl_3$) for compound **1** (δ in ppm, *J* in Hz).

Position	δ_C [a]	δ_H *J* [b]	Position	δ_C [a]	δ_H *J* [b]
1*α*	38.9 (t)	2.48, overlap	14*β*		1.49, overlap
1*β*		1.87, d, *J*= 3.4 Hz	15	220.0 (s)	
2*α*	67.6 (d)	5.17, dd, *J*= 8.8, 2.8 Hz	16	47.6 (s)	2.33, m
3*α*	76.6 (d)	4.80, d, *J*= 2.6 Hz	17	10.6 (q)	1.12, s
4	38.0 (s)		18	26.0 (q)	0.81, s
5*β*	47.7 (d)	1.45, overlap	19	22.4 (q)	1.06, s
6*α*	66.6 (d)	4.97, br d, J=5.6 Hz	20	19.5 (q)	1.43, s
7	36.8 (t)	1.49, overlap	OAc	170.2 (s)	
8	48.3 (s)			169.8 (s)	
9*β*	57.6 (d)	1.22, overlap		169.8 (s)	
10	38.0 (s)			168.6 (s)	2.00, s
11*β*	66.9 (d)	5.28, overlap		21.4 (q)	1.08, s
12*α*	38.8 (t)	1.81, overlap		21.3 (q)	2.07, s
12*β*		1.34, overlap		20.9 (q)	1.91, s
13*α*	34.2 (d)	2.49, m		20.7 (q)	2.10, s
14*α*	37.6 (t)	2.02, overlap			

[a] At 125 MHz, [b] At 500 MHz

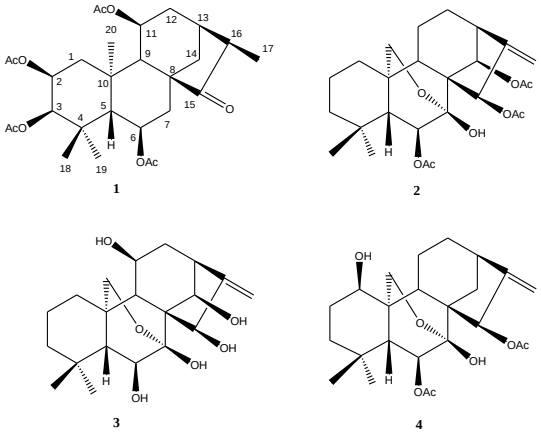

Figure 1. Structures of compounds **1**-**4**.

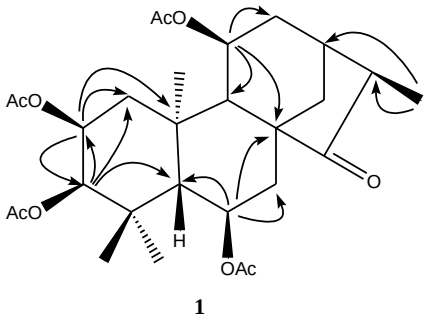

Figure 2. The key HMBC (H→C) correlations of **1**.

3. Results and Discussion

3.1. Structure elucidation

Compound **1** obtained as white amorphous powder. It was assigned the molecular formula $C_{28}H_{40}O_9$ by HRESIMS (*m/z* 543.2579 $[M + Na]^+$). From the ^{13}C-NMR spectra, there were four methyls (δ_C 26.0, 22.4, 19.5, 10.6), four oxy-methines (δ_C 67.6, 76.6, 66.6, 66.9), four methines (δ_C 47.7, 57.6, 34.2 and 47.6), four methylenes (δ_C 38.9, 36.8, 38.8 and 37.6), and four acetoxy groups (δ_C 170.2, 21.4; 169.8, 21.3; 169.8, 20.9; and 168.6, 20.7). Considering the diterpenoids previously isolated from the plant [6], **1** was tentatively presumed to be a 20-non-oxygenated *ent*-kauranoid substituted by four acetoxy groups. The methyl signal at δ_H 1.12 (3H, d) is coupled to C-13 (δ_C 34.2) and C-15 (δ_C 220.0) in the HMBC spectrum (Figure 2), revealing that the C-16 was substituted by a methyl in **1**. The position of the four acetoxy groups were confirmed according to the HMBC spectra. One acetoxy was confirmed at C-2 (δ_C 67.6) by the HMBC correlation of H-2 (δ_H 5.17) with C-1 (δ_C 38.9), C-3 (δ_C 76.6), and C-10 (δ_C 38.0), and the other three acetoxy groups were substituted at C-3 (δ_C 76.6), C-6 (δ_C 66.6) and C-11 (δ_C 66.9) from the HMBC correlations of H-3 (δ_H 4.80) with C-2 (δ_C 67.6), C-1 (δ_C 38.9), and C-5 (δ_C 47.7); H-6 (δ_H 4.97) with C-5 (δ_C 47.7), C-7 (δ_C 36.8), and C-8 (δ_C 48.3); H-11 (δ_H 5.28) with C-12 (δ_C 38.8), C-9 (δ_C 57.6), and C-8 (δ_C 48.3), respectively. The observed ROESY correlations from H-2 to H-19, H-3 to H-20, H-6 to H-19, and H-11 to H-20 showed that the H-1, 3, 6 and 11 was in *a*-orierntation, respectively (Figure 3). The H-16 was deduced to be in *α*-orierntation, according to the Me-17 shown a relatively high field at δ_C 10.6 caused by a steric

compression effect between Me-17 and OAc-11*β*. Thus, the structure of **1** was determined to be 2*β*,3*β*,6*β*,11*β*-tetraacetoxy-*ent*-kaur-16*β*-methy-15-one, and named parvifonin.

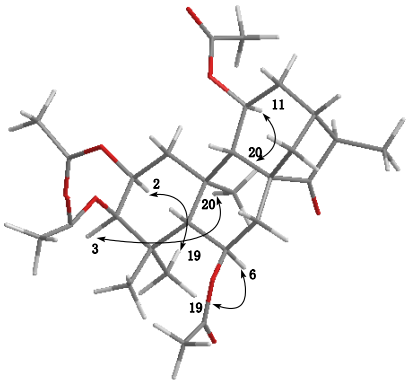

Figure 3. Key ROESY (H↔H) correlations of **1**.

The structure of the known compound hebeirubescensin L (**2**) [7], rabescensin C (**3**) [8], and trichokaurin (**4**) [9] were identified based on spectroscopic methods and literature.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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