A New Asymmetric *ent*-Kauranoid Dimer from *Rabdosia rubescens*

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Abstract: **Objective** To study the *ent*-kaurane diterpenoids from *Rabdosia rubescens*. **Methods** The compounds were isolated by chromatographies and their structures were identified by spectral analyses. **Results** Four compounds were isolated, and they were identified as bisrubescensin E (**1**), 2α,3α,24-trihydroxyurs-12-en-28-oic acid (**2**), 2α,3α,24-trihydroxyurs-12,20-(30)-dien-28-oic acid (**3**), and 6,7-dihydroxycoumarin (**4**). **Conclusion** Compound **1** is a new asymmetric *ent*-kauranoid dimer. Compound **2** is isolated from the plant for the first time. Compounds **3** and **4** are isolated from the plants of *Rabdosia* (Bl.) Hassk for the first time.

Key words: asymmetric *ent*-kauranoid dimer; coumarin; diterpenoid; *Rabdosia rubescens*; triterpenoid
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Introduction

Rabdosia rubescens (Hemsl.) Hara, recorded in *Chinese Pharmacopoeia* 1977, is a perennial herb in genus of *Rabdosia* (Bl.) Hassk (Labiatae), and native to the Yellow River valley of China. It is a famous folk medicine for its antibacterial, anti-inflammatory, and antitumor activities and has been used as an antitumor folk medicine in the treatment of esophageal and cardiac carcinoma in Henan Province, China. The genus of *Rabdosia* (Bl.) Hassk is well known as a rich source of *ent*-kaurane diterpenoids. Many of these diterpenoids display various biological activities, such as anti-bacterial, anti-inflammatory, stomachic, and especially antitumor actions (Ji, Hong, and Gao, 2010). Some *ent*-kaurane derivatives even displayed significant anti-HIV activity (Sun, 2001; Sun, Huang, and Han, 2006).

Our search for bioactive substances from *R. rubescens* has led to the isolation of one asymmetric *ent*-kauranoid dimer, bisrubescensin D (Lu *et al*, 2008) and more than ten other diterpenoids (Lu *et al*, 2008; Lu, Liang, and Chen, 2007). In further study on the minor diterpenoid constituents of this species, we isolated a new asymmetric *ent*-kauranoid dimer, bisrubescensin E (**1**). In this paper, we report the isolation and structure elucidation of the new asymmetric *ent*-kauranoid dimer.

Materials and methods

Plant materials

The aerial parts of *Rabdosia rubescens* (Hemsl.) Hara were collected in Jiyuan county, Henan Province (2004) and identified by Prof. SONG Xue-hua of China Pharmaceutical University. The voucher specimen (20041231) is deposited in Traditional Chinese Medicine Specimen Hall of China Pharmaceutical University.

Instruments

Melting points were measured on an XT—4 Micro Melting-point Apparatus. NMR spectra were recorded on Bruker AV—300 and AV—500 Spectrometers, *J* in Hz, Me_4Si as internal standard, and DMSO-d_6 as solvent. MS spectra were run on Agilent 1100 Series LC/MSD Trap ESI and Agilent 1100 LC/TOF MSD Spectrometers, in *m/z*. All solvents used were of analytical grade (Tianjin Chemical Plant, Tianjin, China). Column chromatography was performed on silica gel (100－200 and 200－300 mesh; Qingdao Marine Chemical Co., Ltd., Qingdao, China), and Sephadex LH-20 (25－100 mm). Thin layer chromatography was performed on silica gel GF_{254} (Yantai Chemical Industrial Institute, Yantai, China).

Extraction and isolation

The air-dried and powdered aerial parts (10 kg)

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were extracted for three times with refluxing 85% EtOH at 80 ℃. The combined extracts were evaporated *in vacuo*, and the resulting residue was suspended in H_2O and subsequently extracted with petroleum ether, $CHCl_3$, and EtOAc. The EtOAc fraction (48 g) was fractionated by column chromatography (silica gel 100－200 mesh, 500 g, $CHCl_3$-MeOH 1:0→0:1) to afford eight fractions (Fr. 1－8). Fr. 3 (10 g) was separated by silica gel (200－300 mesh, 200 g) column chromatography, eluted with petroleum ether-EtOAc (10:1→0:1) to give five sub-fractions (Fr. 3a－3e). Fr. 3d (2.3 g) was repeatedly separated by silica gel (200－300 mesh) column chromatography, and further over Sephadex LH-20 to give compounds **1** (9.9 mg), **2** (15 mg), **3** (13 mg), and **4** (6.5 mg).

Results and discussion

Bisrubescensin E (**1**) was obtained as white powder ($CHCl_3$-MeOH 1:1), mp 236－238 ℃. The (−) ESI-MS *m/z*: 741 $[M-H]^-$ and NMR data indicated the molecular formula of $C_{40}H_{54}O_{13}$. Spraying 10% H_2SO_4 on TLC (silica gel), it displayed the same colour change as other *ent*-kauranoids isolated. In ^{13}C-NMR spectrum, many carbon signals occurred in pairs. Therefore, it was assumed that compound **1** was an *ent*-kauranoid dimer. The chemical structure is shown in Fig. 1.

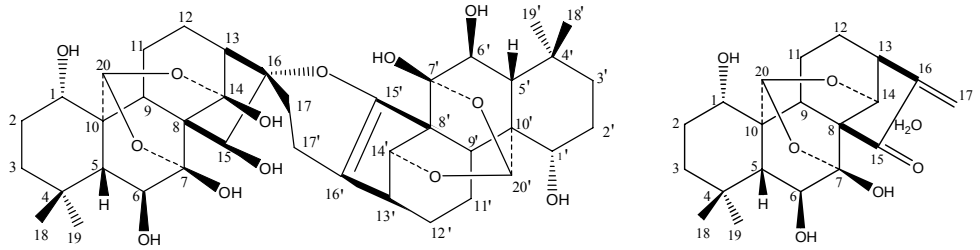

Fig. 1 Structures of fractions a and b of compound 1 and 1c

In ^{1}H-NMR and HSQC spectra, it displayed hydroxyl protons at δ 8.58 (brs), 8.35 (brs), 5.97 (s, 14-OH), 5.38 (d, J = 5.8 Hz), seven proton signals binding with oxygenated tertiary carbons at δ 3.82－3.85 (2H, m, H-1, 1′), 4.04 (2H, d, J = 7.3 Hz, H-6, 6′), 5.38 (1H, d, J = 5.7 Hz, H-14′), 5.74 (1H, s, H-20′), 5.82 (1H, s, H-20), and four methyls at δ 1.00 (3H, s, 18-CH_3), 0.96 (3H, s, 18′-CH_3), 0.91 (6H, s, 19, 19′-CH_3). The ^{13}C-NMR spectrum showed olefin signals at δ 153.0 (C-15′), 112.9 (C-16′), five carbon signals binding with two oxygen at δ 106.6 (C-14), 102.9 (C-7′), 102.8 (C-7), 96.4 (C-20′), 96.1 (C-20); seven carbon signals binding with one oxygen at δ 90.4 (C-16), 73.8 (C-6′), 72.6 (C-6), 72.4 (C-1, 1′), 72.2 (C-15), 71.1 (C-14′) (Table 1). It indicated that the subunits of the dimer **1** were $C_{20,7:20,14}$-diepoxy-*ent*-kauranoids.

By comparison with the NMR data of a known *ent*-kauranoid, ponicidin (**1c**), one of the major constituents of this plant, it was revealed that these two substructures (a, b) of compound **1** were similar to **1c**. The prominent features distinguishing two units in **1** (a, b) from **1c** were the replacement of the α- and β-unsaturated ketone group signals. In part a, they were replaced by an oxygenated tertiary carbon at δ 72.2 (C-15), an oxygenated quaternary carbon at δ 90.4 (C-16), and a methylenes at δ 19.6 (C-17). In part b, they were replaced by an olefinic bond at δ 153.0 (C-15′) and 112.9 (C-16′), and a methylene at δ 16.9 (C-17′). The unsaturation degrees of a and b were both 16. However the 14 degrees of unsaturation required by the molecular formula indicated the presence of an additional ring. The key changes of these charac- ristic signals suggested that subunits a and b were linked by a ring. This was confirmed by the related HMBC and ROESY correlations (Fig. 2).

In HMBC spectrum, the correlations were observed among δ 5.97 (1H, s, OH) with δ_C 106.6 and δ_C 90.4; δ_C 106.6 with 2.67－2.68 (1H, m, H-13) and 1.89－2.00 (1H, m, H-12); δ_C 90.4 with H-13 and δ_H 1.90－2.00 (1H, m, H-17). As a consequence, it indicated that δ_C 106.6 was the signal of C-14, oxygenated quaternary carbon, replaced by OH, and δ_C 90.4 was the signal of C-16, oxygenated quaternary carbon, in a. The correlations of δ_C 112.9 (C-16′) with δ_H 2.79－2.82 (1H, m, H-13′) and 1.54－1.61 (1H, m,

Table 1 ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) data of compound 1 in C_5D_5N (δ, J = Hz)

No.	^{1}H-NMR	^{13}C-NMR	No.	^{1}H-NMR	^{13}C-NMR
1	3.82－3.85 (1H, m)	72.4	1′	3.82－3.85 (1H, m)	72.4
2	1.90－2.00 (2H, m)	30.2	2′	2.29－2.32 (2H, m)	38.2
3	1.24－1.41 (2H, m)	39.9	3′	1.24－1.41 (2H, m)	40.1
4		33.4	4′		33.5
5	1.50 (1H, brs)	63.3	5′	1.50 (1H, brs)	63.8
6	4.04 (1H, d, J = 7.3 Hz)	72.6	6′	4.04 (1H, d, J = 7.3 Hz)	73.8
7		102.8	7′		102.9
8		50.4	8′		51.3
9	2.87 (1H, d, J = 7.3 Hz)	41.3	9′	2.57 (1H, d, J = 6.4 Hz)	42.5
10		48.1	10′		48.3
11	1.47－1.49 (1H, m)	19.1	11′	2.57－2.59 (1H, m)	19.2
	1.90－2.00 (1H, m)			2.25－2.28 (1H, m)	
12	1.90－2.00 (1H, m)	20.3	12′	2.45－2.48 (1H, m)	20.4
	2.57－2.59 (1H, m)			1.90－2.00 (1H, m)	
13	2.67－2.68 (1H, m)	44.1	13′	2.79－2.82 (1H, m)	44.4
14		106.6	14′	5.38 (1H, d, J = 5.7 Hz)	71.1
15	4.92 (1H, d, J = 5.8 Hz)	72.2	15′		153.0
16		90.4	16′		112.9
17	2.29－2.32 (1H, m)	19.6	17′	1.77 (1H, m)	16.9
	1.90－2.00 (1H, m)			1.54－1.61 (1H, m)	
18	1.00 (3H, s)	30.7	18′	0.96 (3H, s)	30.8
19	0.91 (3H, s)	22.9	19′	0.91 (3H, s)	23.0
20	5.82 (1H, s)	96.1	20′	5.74 (1H, s)	96.4
OH	8.58 (brs), 8.35 (brs), 5.97 (s, 14-OH), 5.38 (d, J = 5.8 Hz)				

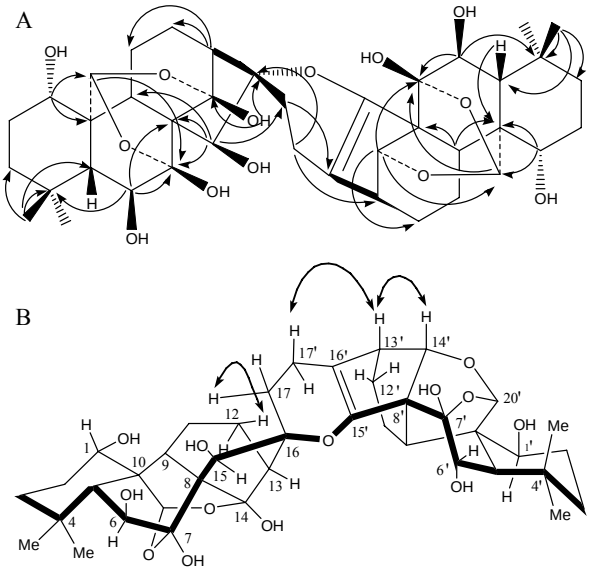

Fig. 2 Key HMBC (H→C) (A) and ROESY (B) correlations of compound 1

H-17′) indicated C-15′, 16′ was olefinic bond, in b. The correlations of C-16′ with H-17 and C-16 with H-17′ indicated the two units were linked by C-17, 17′, and C-16-O-C-15′, a six-membered dihydropyran ring, -C_{16}-C_{17}-$C_{17'}$-$C_{16'}$=$C_{15'}$-O-, identical with enanderinanin J (Na, Li, and Xiang, 2002).

The configuration of C-16 was deduced to be *S* on the basis of the ROESY correlations. NOE cross peak of H-17 with H-12β was observed, which indicated the β-orientation of methylene at C-16. Thus, the structure of compound **1** was determined to be an asymmetric dimer of ponicidine, linked by a six-membered dihydropyran ring, namely bisrubescensin E.

Three known compounds were also isolated from the plant, and their structures were established as two triterpenoids, 2α,3α,24-trihydroxyurs-12-en-28-oic acid

(**2**) (Kojima and Ogura, 1986; Kojima, Tominaga, and Sato, 1987) and 2α,3α,24-trihydroxyurs-12,20-(30)-dien-28-oic acid (**3**) (Kojima, Tominaga, and Sato, 1987), and one coumarin, 6,7-dihydroxycoumarin (**4**) (Salam, Mooney, and Stephen, 1968), respectively, by comparison of the spectroscopic and physical data with those reported in the literature. Among them, compound **2** was isolated from the plant for the first time, and compounds **3** and **4** were isolated from the plants in *Rabdosia* (Bl.) Hassk for the first time.

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Editorial Board of Tianjin Press of Chinese Herbal Medicines and International Symposium on Drug Development

November 4－6, 2011
Jinan, China

Editorial members of four journals published by Tianjin Press of Chinese Herbal Medicines (*Chinese Traditional and Herbal Drugs*, *Chinese Herbal Medicines*, *Drugs & Clinic*, and *Drug Evaluation Research*) met together during November 4－6, 2011 and the International Symposium on Drug Development was also held. Nearly 150 experts namely editorial members, special experts, and reviewers of the four journals attended the meeting.

The 12th Meeting of *Chinese Traditional and Herbal Drugs*, the 3rd get-together of the 1st Editorial Board of *Chinese Herbal Medicines*, and the 2nd Meeting of *Drugs & Clinic* and *Drug Evaluation Research* were held simultaneously. Prof. ZOU Mei-xiang, the editor-in-chief of *Drugs & Clinic* chaired the general meeting. Prof. TANG Li-da, the editor-in-chief of *Chinese Traditional and Herbal Drugs* and President of Tianjin Press of Chinese Herbal Medicines and Prof. LIU Chang-xiao, the academician and the associate editor-in-chief of *Chinese Herbal Medicines*, addressed with the high praise on the editorial work of the four journals. Prof. CHEN Chang-qing, the manager of Tianjin Press of Chinese Herbal Medicines reported on the latest progress of the four journals in the past two years and also expressed great thanks to the experts who have made great contributions to the development of Tianjin Press of Chinese Herbal Medicines.

In the meeting, six experts who have achieved the outstanding results in the related fields both at home and abroad in recent years lectured with the excellent academic reports. The participants have reached a consensus on a great success of the vivid contents in the four journals and orderly work of the Tianjin Press of Chinese Herbal Medicines.

Tianjin Press of Chinese Herbal Medicines