



Bisleuconins A–D: a pair of epimeric *ent*-kauranoid dimers and two new asymmetric analogues isolated from *Isodon leucophyllus*

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ABSTRACT

A phytochemical investigation of *Isodon leucophyllus* led to the isolation of four novel *ent*-kauranoid dimers: bisleuconins A–D (**1**–**4**), and one known compound, rabdoloxin A (**5**). It was interesting that the structures of bisleuconins A (**1**) and B (**2**) were elucidated as a pair of epimeric *ent*-kauranoid dimers with unique linkage pattern C-16→C-17' to connect two monomers. Bisleuconins C (**3**) and D (**4**) were two new asymmetric *ent*-kauranoid dimers. A possible biogenetic pathway of **1** and **2** was also proposed.

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Isodon genus (formerly named *Rabdosia*) is an important genus in *Labiatae* family as it has provided many structurally diverse and bioactive diterpenoids.¹ Over the past 30 years, a series of *ent*-kauranoid dimers with seven kinds of linkage pattern have been found by our group.^{2–8} However, the diterpenoid dimers with epimerization at C-16' and connected by the unique linkage pattern (C-16 with C-17'), have never been reported.

Isodon leucophyllus (Dunn) Kudo is a small shrub mainly distributed in the western area of Sichuan Province and the north-western region of Yunnan Province, People's Republic of China.⁹ Previous research reported the isolation and characterization of 28 diterpenoids (C-20 nonoxygenated and 7,20-epoxy *ent*-kaurane), 6 flavones and one derivative of ionone.^{10–15} In continuation of our research for new diterpenoids with antitumor activities, we have reinvestigated the aerial parts of *I. leucophyllus*, collected in Shangri-La County, Yunnan Province. As a result, a pair of epimeric *ent*-kauranoid dimers (**1** and **2**) and two new asymmetric *ent*-kauranoid dimers (**3** and **4**) along with one known compound, rabdoloxin A (**5**),¹⁶ were isolated from this plant. Herein, we report the isolation, structure elucidation, and their cytotoxicity evaluation, as well as the hypothetically biogenetic pathway of **1** and **2**.

Aerial parts of *Isodon leucophyllus* (Dunn) Kudo were collected and air dried in Shangri-La County of Yunnan Province in August, 2004. The plant material was identified by Professor Xi-Wen Li, and a voucher specimen was deposited in the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences. Powdered aerial parts of *I. leucophyllus* (1.8 kg) were extracted with 70% aq acetone (3 × 6 L) at room temperature for 3 days each time. The extract was evaporated in vacuo to remove acetone, then partitioned between H₂O and EtOAc. The EtOAc extract (78 g) was decolorized with MCI gel, and then chromatographed over a silica gel column (650 g, 100–200 mesh, Qingdao marine chemical factory), eluted with a gradient solvent system [CHCl₃–CH₃COCH₃ (1:0, 9:1, 8:2, 7:3, 2:1, 1:1, 0:1)] to afford fractions A–G, monitoring by TLC (volume of each collection was 1000 mL). Fraction C (8:2, 11 g) was submitted to CC over a RP-18 column (200 g, 40–63 μm, Merck Company, 30%→100% MeOH–H₂O) to give fractions C1–C5, monitoring by TLC (volume of each collection was 250 mL). In the sixth, seventh and eighth bottles of elution solvent belonging to fraction C1, compound **5** (3.1 g) was separated as needle crystals. Fraction C2 (2.3 g) was subjected to silica gel CC (200–300 mesh, 40 g) eluting with a gradient solvent system of light petroleum–CH₃COCH₃ (1:0 to 0:1, volume of each collection was 50 mL). Compounds **1** (3 mg) and **2** (5 mg) were isolated by semi-preparative HPLC (20% CH₃CN–H₂O, λ_{max} = 202 nm) from the mixture of the fourth–eighth bottles of elution solvent. Compounds **3** (12 mg) and **4** (26 mg) were purified from fraction F

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(1:1) by reverse phase silica gel CC on RP-18, followed by semi-preparative HPLC (55% MeOH–H₂O, λ_{max} = 202 nm, and 35% THF–H₂O, λ_{max} = 210 nm, respectively.)

Compound **1** was isolated as white amorphous powder, and showed a quasi-molecular ion peak at m/z 783.3926 ($[M+Na]^+$, calcd 783.3931) in its HRESIMS, corresponding to a molecular formula C₄₁H₆₀O₁₃ requiring 12 degrees of unsaturation.¹⁷ The IR spectrum of **1** showed absorption bands for hydroxyl (3416 cm^{−1}), methyl (2931 cm^{−1}), carbonyl (1738, 1705 cm^{−1}) groups. The ¹H, ¹³C, and DEPT NMR data (Table 1) showed 41 carbon resonances due to four tertiary methyls, one methoxyl, twelve methylenes (including three oxygenated ones), thirteen methines (including six oxygenated ones), and eleven quaternary carbons (including four carbonyl groups). The carbon signals of **1** mostly appeared in pairs, which showed that compound **1** could be a diterpenoid dimer.

Comparing the ¹³C NMR data of compound **1** with those of rabdoloxin A (**5**) (the main constituent in *I. leucophyllus*) revealed that the two monomers (**1a** and **1b**, Table 1) of **1** possessed similar structure to rabdoloxin A except that the conjugated double bond in **5** disappeared in **1a** and **1b**, which were replaced by one quaternary carbon (δ_C 54.7, s, C-16), two methylenes (δ_C 74.3, t, C-17; δ_C 31.2, t, C-17'), and one methine (δ_C 45.9, d, C-16'). The above assignments were established on the basis of the ¹H–¹H COSY correlations of H-13'/H-16'/H-17' (Fig. 1), together with the HMBC correlations of H-17/C-15, C-16; H-17'/C-15; and H-16'/C-16. All those key correlations suggested that the subunits **1a** and **1b** were connected by a single carbon–carbon bond (C-16→C-17'), which was an unique linkage pattern in *ent*-kauranoid dimers. One methoxyl was located at C-17 as it had the HMBC correlation with C-17. ROESY experiment was applied to establish the relative stereochemistry of **1**. Correlations from H-17 to H-12 β and from H-17' to H-12' β indicated that C-17 in **1a** and C-17' in **1b** adopted β -orientations (Fig. 1). Correlations of H-16'/H-13' α and H-13 α gave H-16' an α -orientation. Therefore, compound **1** was finally elucidated

Table 2

¹H and ¹³C NMR data of compound **2**^a (in C₅D₅N, δ in ppm, J in Hz)

No.	2a		No.	2b	
	δ_H	δ_C		δ_H	δ_C
1	1.35 (m, overlap) 1.95 (m, overlap)	39.9 t	1'	1.20 (m, overlap) 2.23 (m, overlap)	39.9 t
2	1.43 (m)	18.3 t	2'	1.43 (m)	18.5 t
3	1.60 (m, overlap) 1.32 (m, overlap) 1.82 (m)	35.4 t	3'	1.60 (m, overlap) 1.32 (m, overlap) 1.82 (m)	35.4 t
4		41.5 s	4'		41.5 s
5	1.92(m, overlap)	46.3 d	5'	1.92 (m, overlap)	46.3d
6	2.05–2.25 (m) 2.45 (m)	29.8 t	6'	2.05–2.25 (m) 2.45 (m)	29.8 t
7	4.95 (br d)	73.4 d	7'	4.95 (br d)	74.3 d
8		61.3 s	8'		60.7 s
9	2.30 (s)	69.7 d	9'	2.32 (s)	69.7 d
10		38.4 s	10'		38.3 s
11		208.8 s	11'		210.0 s
12	4.60 (s)	75.2 d	12'	4.82 (s)	78.2 d
13	3.55 (s, overlap)	51.0 d	13'	3.08 (s, overlap)	53.7 d
14	6.31 (s)	73.8 d	14'	6.45 (s)	73.6 d
15		221.8 s	15'		221.7 s
16		54.6 s	16'	2.91 (d, 6.2)	45.6 d
17	3.58 (m, overlap) 3.69 (m, overlap)	73.4 t	17'	3.07 (m, overlap) 3.75 (m, overlap)	41.0 t
18	3.31 (br d) 3.72 (m, overlap)	71.2 t	18'	3.31 (br d) 3.72 (m, overlap)	71.2 t
19	0.77 (s)	18.2 q	19'	0.83 (s)	18.2 q
20	1.58 (s)	18.9 q	20'	1.72 (s)	18.9 q
17-OMe	3.05(s)	58.2 q			

^a ¹H and ¹³C NMR data of compound **2** were recorded at 500 and 125 MHz.

as an asymmetric *ent*-kauranoid dimer, and named as bisleuconin A.

Interestingly, we also found one epimeric *ent*-kauranoid dimer (compound **2**) of **1** in the process of isolation. Compound **2** is a

Table 1

¹H and ¹³C NMR data of compounds **1a** and **5b** (in C₅D₅N, δ in ppm, J in Hz)

No.	1a		No.	1b		No.	5
	δ_H	δ_C		δ_H	δ_C		δ_C
1	1.61 (m, overlap) 1.93 (m, overlap)	39.8 t	1'	1.30 (m, overlap) 2.25 (m, overlap)	40.0 t	1	39.8 t
2	1.43 (m)	18.3 t	2'	1.43 (m)	18.5 t	2	18.3 t
3	1.60 (m, overlap) 1.31 (m, overlap) 1.85 (d, 12.6)	35.0 t	3'	1.60 (m, overlap) 1.31 (m, overlap) 1.85 (d, 12.6)	35.0 t	3	35.4 t
4		41.5 s	4'		41.0 s	4	41.2 s
5	1.9 (m, overlap)	46.2 d	5'	1.9 (m, overlap)	46.8 d	5	46.2 d
6	2.15 (m) 2.43 (m)	29.4 t	6'	2.15 (m) 2.43 (m)	29.2 t	6	29.6 t
7	4.91 (br s)	73.9 d	7'	4.91 (br s)	73.8 d	7	73.3 d
8		61.1 s	8'		59.4 s	8	60.2 s
9	2.35 (s)	69.6 d	9'	2.30 (s)	70.4 d	9	70.2 d
10		38.4 s	10'		38.3 s	10	38.4 s
11		208.6 s	11'		209.4 s	11	208.7 s
12	4.55 (s)	75.2 d	12'	4.96 (s)	73.6 d	12	78.9 d
13	3.27 (s)	53.0 d	13'	3.61 (s, overlap)	50.2 d	13	53.4 d
14	6.31 (s)	73.6 d	14'	6.45 (s)	71.9 d	14	71.2 d
15		220.9 s	15'		219.9 s	15	206.7 s
16		54.7 s	16'	4.07 (m)	45.9 d	16	144.9 s
17	3.61 (AB d, overlap) 3.82 (AB d, 7.4)	74.3 t	17'	2.75 (m) 3.31 (m, overlap)	31.2 t	17	122.6 t
18	3.35 (m, overlap) 3.70 (m)	71.2 t	18'	3.35 (m, overlap) 3.70 (m)	71.2 t	18	71.2 t
19	0.77 (s)	18.1 q	19'	0.83 (s)	18.2 q	19	18.2 q
20	1.58 (s)	18.9 q	20'	1.70 (s)	19.0 q	20	18.9 q
17-OMe	2.96 (q)	57.9					

^a ¹H and ¹³C NMR data of compound **1** were recorded at 500 and 125 MHz.

^b ¹³C NMR data of compound **5** were recorded at 125 MHz.

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