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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.

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 H_2O and EtOAc. The EtOAc extract (78 g) was decolored 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 [$CHCl_3$–CH_3COCH_3 (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–H_2O) 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–CH_3COCH_3 (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% CH_3CN–H_2O, 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–H_2O, λ_{max} = 202 nm, and 35% THF–H_2O, 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 $C_{41}H_{60}O_{13}$ 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 ^{1}H, ^{13}C, 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 ^{13}C 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 ^{1}H–^{1}H 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-12β 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-13α gave H-16′ an α-orientation. Therefore, compound **1** was finally elucidated 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 2
^{1}H and ^{13}C NMR data of compound **2** [a] (in C_5D_5N, 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) 1.60 (m, overlap)	18.3 t	2′	1.43 (m) 1.60 (m, overlap)	18.5 t
3	1.32 (m, overlap) 1.82 (m)	35.4 t	3′	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] ^{1}H and ^{13}C NMR data of compound **2** were recorded at 500 and 125 MHz.

Table 1
^{1}H and ^{13}C NMR data of compounds **1**[a] and **5**[b] (in C_5D_5N, 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) 1.60 (m, overlap)	18.3 t	2′	1.43 (m) 1.60 (m, overlap)	18.5 t	2	18.3 t
3	1.31 (m, overlap) 1.85 (d, 12.6)	35.0 t	3′	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] ^{1}H and ^{13}C NMR data of compound **1** were recorded at 500 and 125 MHz.
[b] ^{13}C NMR data of compound **5** were recorded at 125 MHz.

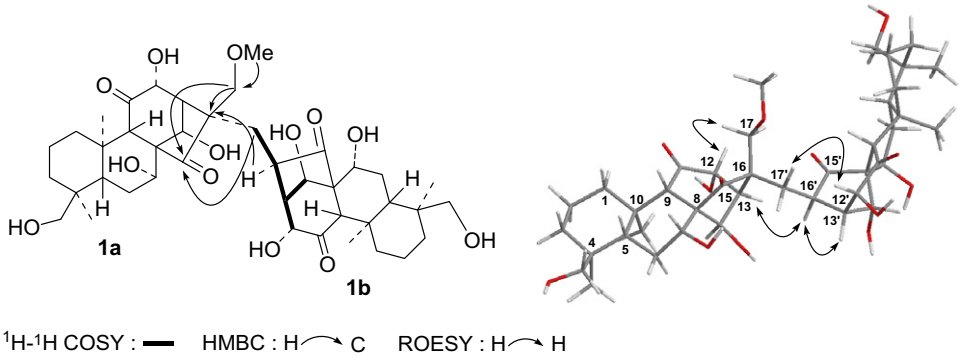


Figure 1. Selected ^{1}H–^{1}H COSY, HMBC and ROESY correlations of **1**.

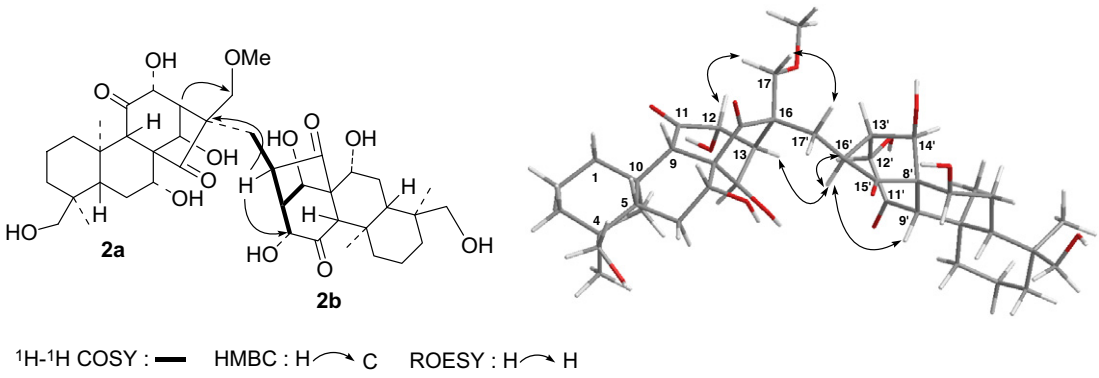


Figure 2. Selected ^{1}H–^{1}H COSY, HMBC and ROESY correlations of **2**.

Table 3
^{1}H and ^{13}C NMR data of compounds **3** [a] (in C_5D_5N, δ in ppm, J in Hz)

No.	3a		No.	3b	
	δ_H	δ_C		δ_H	δ_C
1	1.30 (m, overlap) 1.95 (m, overlap)	39.7 t	1′	1.30 (m, overlap) 1.95 (m, overlap)	39.7 t
2	1.40 (m, overlap) 1.58 (m)	18.5 t	2′	1.40 (m, overlap) 1.58 (m)	18.7 t
3	1.32 (m, overlap) 1.83 (m, overlap)	35.1 t	3′	1.32 (m, overlap) 1.70 (m, overlap)	35.2 t
4		41.4 s	4′		41.4 s
5	1.93(m, overlap)	46.5 d	5′	1.87 (m, overlap)	46.8d
6	2.13 (m) 2.45 (m)	29.3 t	6′	2.13 (m) 2.32 (m)	30.1 t
7	4.85 (br d)	74.3 d	7′	4.75 (br d)	72.8 d
8		60.8 s	8′		54.6 s
9	2.32 (s)	71.1 d	9′	3.06 (s)	68.0 d
10		38.3 s	10′		38.3 s
11		211.8 s	11′		208.7 s
12	4.42 (s)	74.1 d	12′	4.27 (s)	74.5 d
13	3.25 (s, overlap)	52.4 d	13′	3.55 (s, overlap)	54.1 d
14	6.45 (s)	72.6 d	14′	6.1 (s)	74.5 d
15		215.9 s	15′		155.1 s
16		82.1 s	16′		108.3 s
17	1.56 (m, overlap) 2.17 (m, overlap)	25.2 t	17′	1.42 (m, overlap) 2.08 (m, overlap)	40.5 t
18	3.25 (m, overlap) 3.67 (AB, d)	71.0 t	18′	3.25 (m, overlap) 3.51 (AB, d)	71.2 t
19	0.78 (s)	18.0 q	19′	0.83 (s)	18.1 q
20	1.65 (s)	18.9 q	20′	1.69 (s)	19.2 q

[a] ^{1}H and ^{13}C NMR data of compound **3** were recorded at 400 and 100 MHz.

white amorphous powder. Its molecular formula $C_{41}H_{60}O_{13}$ was determined by the positive HRESIMS (m/z 783.3919 ([M+Na]$^+$, calcd 783.3931), with 12 degrees of unsaturation.[18] Carefully comparing the carbon signals of **2** and **1**, we found that most of two monomer's data were identical except for the obvious downfield chemical shift (about 10 ppm) of one methylene (C-17′, δ_C 41.0;) in **2b** in comparison with the counterpart (C-17′, δ_C 31.2) in **1b**. This great difference implied that compound **2** might be an epimeric isomer of **1**. The linkage pattern of **2** were same as **1** which was elucidated on the basis of HMBC correlations of H-16′/C-16, H-16′/C-12′, and H-13/C-17′ and ^{1}H–^{1}H COSY correlations of H-12′/H-13′/H-14′; H-13′/H-16′/H-17′ (Fig. 2). The key ROESY correlations of H-16′/H-9′β, H-16′/H-12′β, H-16′/H-13, H-17/H-12β, and H-17/H-17′ established the β-orientation of H-16′ (Fig. 2). Thus, compound **2** was eventually determined to be an isomer of **1** epimerized at C-16′, and named as bisleuconin B.

Compound **3**, obtained as white amorphous powder, has the molecular formula $C_{40}H_{56}O_{12}$ surpported by the HRESIMS of the [M+Na]$^+$ ion peak at m/z (751.3672, calcd 751.3669), corresponding to 13 degrees of unsaturation.[19] IR spectrum of **3** showed absorption bands for hydroxyl (3377 cm^{-1}), methyl (2932 cm^{-1}), carbonyl (1752, 1697 cm^{-1}) groups. Compound **3** was also presumed to be an asymmetric *ent*-kauranoid dimer as its carbon signals exhibited the characteristics (appearing in pairs) of asymmetric *ent*-kauranoid dimmers like compounds **1** and **2**. Detailed comparison of the ^{13}C NMR data of subunits **3a** and **3b** with those of **5** (Table 3), we found that they resembled each other except for the absence of two *exo*-methylenes conjugated with cyclopentanone in **5**. The presence of tetrasubstituted double bond [δ_C 155.1 (s, C-15′), 108.3 (s, C-16′)], two additional methylenes [δ_C 24.8 (t, C-17), 39.7 (t, C-17′)] and an abnormal oxygenated quaternary carbon [δ_C 82.1 (s, C-16)], combining with the 13 degrees of unsaturation of compound **3**, suggested that it was an asymmetric dimmer linked by a six-membered dihydropyran ring

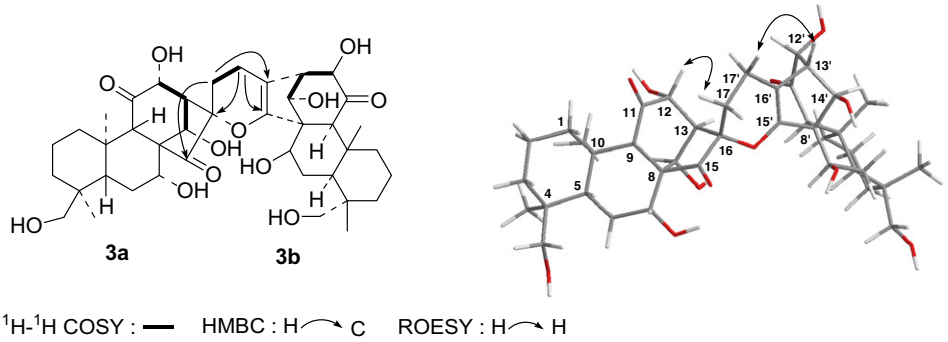


Figure 3. Selected ^{1}H–^{1}H COSY, HMBC and ROESY correlations of **3**.

Table 4
^{1}H and ^{13}C NMR data of compounds **4**[a] (in C_5D_5N, δ in ppm, J in Hz)

No.	4a		No.	4b	
	δ_H	δ_C		δ_H	δ_C
1	1.13 (m, overlap)	39.6 t	1′	1.30 (m, overlap)	39.7 t
	2.05 (m, overlap)			1.91 (m, overlap)	
2	1.41 (br d)	18.2 t	2′	1.32 (m, overlap)	18.9 t
	1.58 (m)			1.69 (m, overlap)	
3	1.31 (m, overlap)	35.5 t	3′	1.12 (m, overlap)	41.9 t
	1.80 (m)			1.32 (m, overlap)	
4		40.9 s	4′		33.7 s
5	1.91(m, overlap)	46.7 d	5′	1.02 (d, 11.6)	53.7d
6	2.13 (m, overlap)	29.7 t	6′	1.91 (t)	30.7 t
	2.43 (m, overlap)			2.17 (t)	
7	4.86 (br d)	74.1 d	7′	4.17 (br d)	78.7 d
8		60.4 s	8′		56.7 s
9	2.30 (s)	70.2 d	9′	1.69 (s, overlap)	64.1 d
10		38.3 s	10′		38.7 s
11		209.0 s	11′	4.30 (s)	73.6 d
12	4.43 (s)	74.1 d	12′	4.52 (s)	75.2 d
13	3.32 (br d)	47.8 d	13′	3.13 (s)	59.9 d
14	6.34 (s)	71.7 d	14′	5.56 (s)	76.9 d
15		220 s	15′	5.77 (s)	133.4 d
16	3.68 (m)	48.0 d	16′		143.1 s
17	1.71 (m)	23.7 t	17′	2.43 (m, overlap)	29.2 t
	2.43 (m, overlap)			2.52 (m, overlap)	
18	3.28 (AB, d, 2.7)	71.2 t	18′	1.31 (m, overlap)	33.5 t
	3.64 (AB, d, 2.7)			1.80 (m)	
19	0.83 (s, overlap)	18.1 q	19′	0.77 (s)	21.9 q
20	1.69 (s)	18.9 q	20′	1.47 (s)	17.2 q

[a] ^{1}H and ^{13}C NMR data of compound **4** were recorded at 400 and 100 MHz.

like bisrubescensin C.[3] This deduction was confirmed by a series of key HMBC correlations of H-17′ with C-16, C-15′, and C-16′; H-17 with C-15 and C-16′; and of H-13 with C-11 and C-15. And ^{1}H–^{1}H COSY correlations of H-12′/H-13′/H-14′, H-12/H-13/H-14, and H_2-17/H_2-17′ further proved the above conclusion (Fig. 3). The stereochemistry of compound **3** was established on the basis of ROESY correlations of H-17 with H-12β and H-17′ with H-13′α, which indicated that the single carbon bond (C-16→C-17) adopted β-orientation in subunit **3a** (Fig. 3). Subsequently, the structure of compound **3** was elucidated as shown in Figure 3, and named as bisleuconin C.

Compound **4** was also an asymmetric *ent*-kauranoid dimmer, isolated as white amorphous powder, having the molecular formula $C_{40}H_{60}O_{10}$ supported by HRESIMS of the $[M+Na]^+$ ion peak at *m/z* 723.4093 ($[M+Na]^+$, calcd 723.4084) with 11 degree of unsaturation.[20] The IR spectrum of **4** showed absorption bands for hydroxyl (3424 cm^{-1}), methyl (2928 cm^{-1}), carbonyl (1736, 1704 cm^{-1}) groups. Carefully comparing the chemical shifts of carbon signals of subunits **4a** and **4b** with those of **5** (Table 4), we found that most of the data of **4a** were identical to those of **5**, but **4b** was one C-19,20-non-oxygenated-*ent*-kaurane diterpenoid. The absence of two *exo*-methylenes conjugated with cyclopentanone in **4a** and **4b**, one trisubstituted double bond [δ_C 143.1 (s, C-16′), 133.4 (d, C-15′)], two additional methylenes [δ_C 23.7 (t, C-17), 29.2 (t, C-17′)], and one methine [δ_C 48.0 (d, C-16)], along with the query of 11 degrees of unsaturation, suggested that compound **4** was an asymmetric dimmer linked by a single carbon bond (C-17→C-17′) like bisrubescensin B.[3] This linkage pattern was confirmed by the HMBC spectrum: H-17′ correlated to C-16, C-13′, C-15′, and C-16′; H-16 correlated to C-13, C-17, and C-17′. The ^{1}H–^{1}HCOSY correlations of H-13/H-16, H-16/H_2-17, and H_2-17/H_2-17′ further confirmed the above

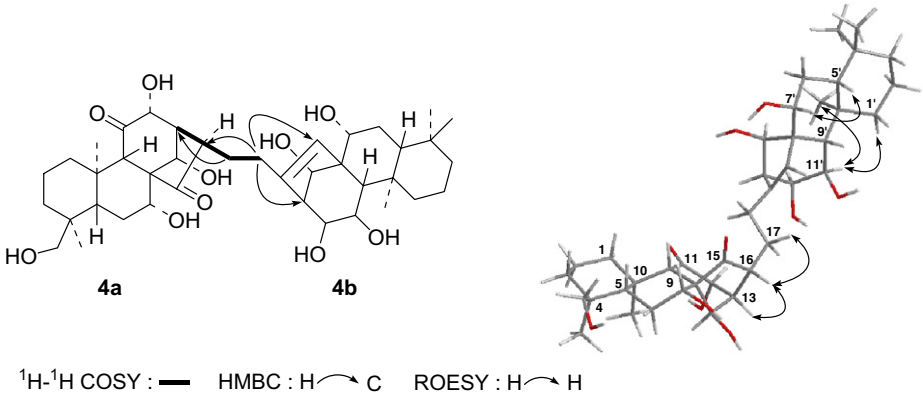
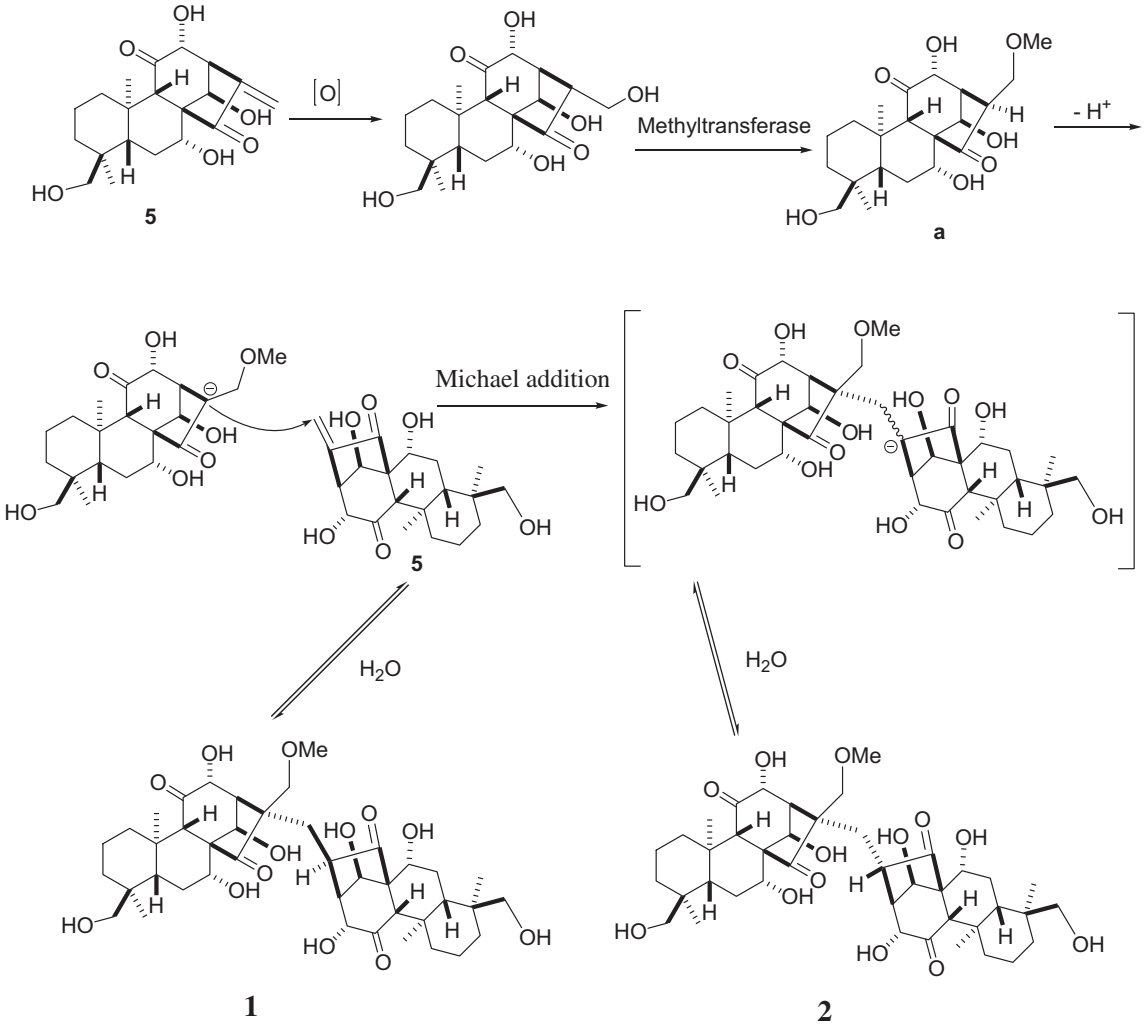


Figure 4. Selected ^{1}H–^{1}H COSY, HMBC and ROESY correlations of **4**.



Scheme 1. Proposed biogenetic pathway for Compounds **1** and **2**.

deduction. The ROESY correlations of H-16/H-13α and H-16/H-17 suggested that H-16 was α-oriented in subunit **4a**. Similarly, H-7′, H-11′, and H-12′ in subunit **4b** were determined to be β, α, and α-orientation, respectively according to the ROESY correlations of H-7′/H-5′β, H-11′/H-1′α, H-11′/H-20′α, H-12′/H-13′α, and H-12′/H-11′. Therefore, the structure of compound **4** was elucidated as shown in Figure 4, and it was named as bisleuconin D.

Bisleuconins A and B (**1** and **2**) were the first example of epimeric *ent*-kaurane diterpenoid dimers (epimerized at C-16′) with the unique linkage pattern from C-16 to C-17′. A plausible biosynthetic pathway for **1** and **2** was proposed as shown in Scheme 1. Compound **5** was oxidized at C-17 and got methylated successively to form the monomer **a**. Then, a Michael addition reaction conducted by providing C-16 in monomer **a** to attack C-17′ in another **5** to form single carbon bond C-16→C-17′. Finally, the epimerization occurred when the carbon anion located at C-16′ got one proton from water and resulted in the forming dimmers **1** and **2**.

Compound **1–4** were evaluated for their cytotoxicity against three human tumor cell lines (HT-29, BEL-7402 and SK-OV-3), using the sulforhodamine B (SRB) method, as reported previously.[21] All of the dimers were inactive with IC_{50} values of >100 μM.

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Supplementary data

Supplementary data (experimental details and MS, ESIMS, 1D, and 2D NMR spectra of bisleuconins A–D) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.08.133.

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17. Bisleuconin A (**1**): white amorphous powder; $[\alpha]_D^{22.5}$ +27.62 (*c* 0.00156 g/ml, MeOH); UV (MeOH) λ_{max} = 203.2 nm, (log ε = 3.70); IR (KBr) cm^{-1}: 3416.4, 2931.3, 2875.4, 1737.9, 1705.7, 1639.2; ESI MS: *m*/*z* 783 ([M+Na]$^+$); HR-ESI MS: *m*/*z* 783.3926 ([M+Na]$^+$), corresponding to a molecular formula $C_{41}H_{60}O_{13}Na$ (calcd 783.3931), for ^1H, ^{13}C NMR data, see Table 1.
18. Bisleuconin B (**2**):white amorphous powder; $[\alpha]_D^{22.5}$ +13.16 (*c* 0.00152 g/ml, MeOH); UV (MeOH) λ_{max} = 203.2 nm, (log ε = 3.71); IR (KBr) cm^{-1}: 3395.6, 2928.8, 2872.9, 1727.8, 1689.8, 1641.1; ESI MS: *m*/*z* 783 ([M+Na]$^+$); HR-ESI MS: *m*/*z* 783.3919 ([M+Na]$^+$), corresponding to a molecular formula $C_{41}H_{60}O_{13}Na$ (calcd 783.3931), for ^1H, ^{13}C NMR data, see Table 2.
19. Bisleuconin C (**3**): white amorphous powder; $[\alpha]_D^{17.4}$ −1.94 (*c* 0.00258 g/ml, MeOH); UV (MeOH) λ_{max} = 202.2 nm, (log ε = 4.03); IR (KBr) cm^{-1}: 3377.5, 2932.5, 2875.1, 1752.3, 1697.1, 1639.7; ESI MS: *m*/*z* 751 ([M+Na]$^+$); HR-ESI MS: *m*/*z* 751.3672 ([M+Na]$^+$), corresponding to a molecular formula $C_{40}H_{56}O_{12}Na$ (calcd 751.3669), for ^1H, ^{13}C NMR data, see Table 3.
20. Bisleuconin D (**4**): white amorphous powder; $[\alpha]_D^{16.2}$ −17.55 (*c* 0.00229 g/ml, MeOH); UV (MeOH) λ_{max} = 204.0 nm, (log ε = 3.78); IR (KBr) cm^{-1}: 3423.7, 2928.3, 2872.1, 1736.2, 1704.7, 1636.7; ESI MS: *m*/*z* 723 ([M+Na]$^+$); HR-ESI MS: *m*/*z* 723.4093 ([M+Na]$^+$), corresponding to a molecular formula $C_{40}H_{60}O_{10}Na$ (calcd 723.4084), for ^1H, ^{13}C NMR data, see Table 4.
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