$C_{30}H_{38}O_7$ $[M + H]^+$ (5), 493 $[M - H_2O + H]^+$ (6), 461 (10), 425 (9), 391 (14), 369 (20), 299 (30), 277 (85), 185 (100); ^{1}H-NMR (Table **1**); ^{13}C-NMR data (Table **2**).

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Diterpenoids from *Isodon leucophyllus*[1]

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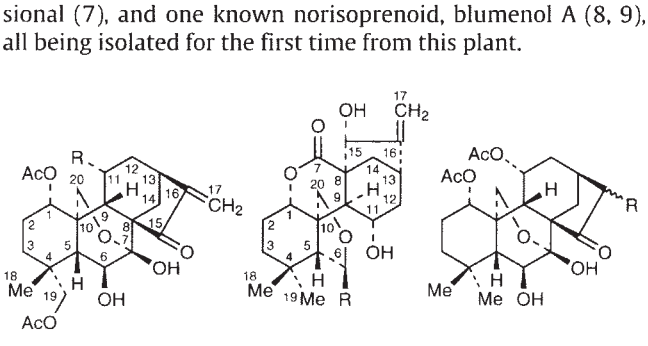
Abstract: **The structures of two new *ent*-kaurane diterpenoids and two derivatives of shikokianin isolated from leaves of *Isodon leucophyllus* were elucidated by 1D and 2D NMR techniques as 11α-acetoxyeffusanin D (1), 6-acetylepinodosinol (2), 16β-ethoxymethyleneshikokianin (3), and 16α-ethoxymethyleneshikokianin (4).**

Isodon leucophyllus (Dunn) Kudo, distributed in the northwest area of Yunnan Province of China, has been used as a folk medicine by local practitioners for antibacterial and anti-inflammatory purposes (1). Previous chemical investigation has resulted in the isolation of four *ent*-kaurene diterpenoids (2). Our recent study of this plant led to the isolation of two new diterpenoids (**1**, **2**) and two derivatives of shikokianin (**3**, **4**) together with six known diterpenoids, longikaurins D – F (3, 4), effusanin D (**5**) (5), shikokianin (**7**) (6), and rabdolasional (7), and one known norisoprenoid, blumenol A (8, 9), all being isolated for the first time from this plant.



1 R = OAc
5 R = H

2 R = OAc
6 R = OH

3 R = β-CH_2OEt
4 R = α-CH_2OEt
7 R = exo-methylene

Compound **1** was determined as $C_{26}H_{34}O_{10}$ ($[M]^+$ *m/z* 506.2157) by HRMS. Its mass spectrum revealed that the molecular ion (*m/z* 506) is 58 amu higher than that of effusanin D (**5**) (5). The ^{1}H-, ^{13}C-, and DEPT-NMR spectra of **1** were very similar to those of **5**. The only difference was that **1** had a methine signal at δ 4.84 and one more acetyl group. The COLOC spectrum of **1** indicated that the methine signal at δ 4.84 correlated with the signals of C-8 (δ 58.0), C-13 (δ 33.4) and an acetyl carbonyl (δ 169.7), respectively. Thus, the methine should be assigned to H-11, with an attached acetyl group at C-11. The relative stereochemistry of C-11-OAc was

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assigned an α-orientation on the basis of the broad multiplet at 4.84 ppm. The relative configurations of the other substituents were elucidated on the basis of comparison of the ^{13}C- and ^{1}H-NMR data with **5**. Therefore, **1** was identified as 11α-acetoxyeffusanin D.

Compound **2** was determined as $C_{22}H_{30}O_7$ by HRMS. Its mass spectrum showed that the molecular ion (*m/z* 406) is 42 amu greater than that of epinodosinol (**6**) (10–12). Its ^{1}H-, ^{13}C-, and DEPT-NMR spectra were very similar to those of **6**, suggesting the same diterpene skeleton. The only difference was that **2** had one more acetyl group. In the COLOC spectrum of **2**, the C-H long range correlation of H-6 (δ 4.71) with an acetyl carbonyl (δ 170.9) indicated that the acetyl group is attached to C-6. The relative configurations of the substituents were elucidated on the basis of comparison of the ^{13}C- and ^{1}H-NMR data with **6**. Therefore, **2** was deduced to be 6-acetylepinodosinol.

For compound **3**, the HRMS gave a molecular formula of $C_{26}H_{38}O_9$. The ^{1}H-, ^{13}C-, and DEPT-NMR spectra of **3** were typical for an epoxy-*ent*-kauranoid with four substituents besides additional ethoxy group. Further study of its ^{1}H- and ^{13}C-NMR data revealed a close resemblance to those of shikokianin (**7**) (6), except for the D-ring. Instead of the exomethylene signals in **7**, the signals for a methine (δ 56.1, C-16) and an oxygenated methylene (δ 67.1, C-17) were observed in the ^{13}C-NMR spectrum of **3**. In ^{13}C-^{1}H COSY, and COLOC spectra of **3**, the correlations of H-16 (δ 2.90) / C-13 (δ 29.1) and H-16 (δ 2.90) / C-17 (δ 67.1) confirmed the presence of the ethoxymethylene function at C-16. The ethoxymethylene group was assigned a β-orientation deduced from the unusual upfield shift (from δ 37.7 in **7** to 28.9 in **3**) of C-12, caused by a γ-effect of the ethoxymethylene group (13, 14). Therefore, **3** was determined as 16β-ethoxymethyleneshikokianin.

Compound **4** had the same molecular formula as **3** from HRMS. Further studies on its ^{1}H-, ^{13}C-, and DEPT-NMR spectra showed that spectral characters were very similar to those of **3** and the differences were that the chemical shift of C-12 changed from δ 28.9 in **3** to δ 37.9 in **4**, and C-14 changed from δ 28.6 in **3** to δ 25.8 in **4**. Besides, the C-17 signal at δ 69.7 in **4** had a downfield shift of 2.6 ppm compared with **3** (C-17 at δ 67.1), indicating that **4** was a C-16 epimer of **3**. Therefore, **4** was deduced as 16α-ethoxymethyleneshikokianin.

Although we do not detect **3** and **4** in the solution of shikokianin in EtOH after refluxing for 3 h, they may be artifacts of shikokianin formed during EtOH extraction.

Table 1 The ^{13}C-NMR data of **1–4**.

Carbon	1[a]	2[b]	3[a]	4[c]
1	75.8 (d)	76.5 (d)	76.6 (d)	76.4 (d)
2	24.6 (t)	23.2 (t)	25.4 (t)	25.0 (t)
3	32.8 (t)	36.2 (t)	39.0 (t)	38.8 (t)
4	36.3 (s)	30.8 (s)	33.7 (s)	33.4 (s)
5	58.3 (d)	52.7 (d)	58.9 (d)	57.6 (d)
6	73.3 (d)	109.3 (d)	75.1 (d)	74.7 (d)
7	94.9 (s)	175.2 (s)	95.8 (s)	94.6 (s)
8	58.0 (s)	52.9 (s)	59.7 (s)	58.3 (s)
9	53.1 (d)	41.9 (d)	51.6 (d)	53.3 (d)
10	41.3 (s)	50.6 (s)	41.4 (s)	41.4 (s)
11	69.3 (d)	66.8 (d)	70.2 (d)	69.4 (d)
12	37.7 (t)	41.0 (t)	28.9 (t)	37.9 (t)
13	33.4 (d)	35.9 (d)	29.1 (d)	29.4 (d)
14	25.7 (t)	34.3 (t)	28.6 (t)	25.8 (t)
15	207.2 (s)	77.2 (d)	221.2 (s)	220.0 (s)
16	151.3 (s)	155.2 (s)	56.1 (d)	57.8 (d)
17	119.6 (t)	109.3 (t)	67.1 (t)	69.7 (t)
18	28.7 (q)	32.4 (q)	34.1 (q)	33.8 (q)
19	66.8 (t)	23.5 (q)	22.7 (q)	22.8 (q)
20	65.1 (t)	72.7 (t)	64.9 (t)	64.9 (t)
OAc	170.9	170.9	170.5	170.3
	170.8	21.1	170.1	169.7
	169.7		22.0	22.0
	22.0		21.8	21.6
	21.5			
	20.9			
OCH_2CH_3			66.5	66.7
			15.2	15.0

[a] Recorded in $CDCl_3$.
[b] Recorded in $CDCl_3$-CD_3OD (10:1).
[c] Recorded in pyridine-d_5.

Materials and Methods

M.p.: uncorr; Optical rotations: Horiba Sepa-300 polarimeter; UV: Shimadzu UV-210A; IR: Perkin-Elmer 577; NMR: Bruker AM-400; EI, HRMS: VG Auto Spec 3000 (70 eV). CC: silica gel. The leaves of *Isodon leucophyllus* (Dunn) Kudo (Labiatae) were collected in Lijiang county, Yunnan Province, China, in August, 1996, and identified by Prof. H.-W. Li of Kunming Institute of Botany, Academia Sinica, where a voucher specimen (KIB 96-08-01, Lin) is deposited.

Dried and powdered leaves of the plant (6.0 kg) were extracted with 95% EtOH (2000 ml × 3) by refluxing for 2 h and then the solvent was removed *in vacuo*. The resultant extract was suspended in H_2O (1000 ml) and extracted with petrol and EtOAc (1000 ml × 3), respectively. The EtOAc extract (200 g) was subjected to CC (12 × 150 cm, 2 kg, 200–300 mesh), eluted with $CHCl_3$-Me_2CO gradient (from $CHCl_3$ to Me_2CO, each 1000 ml) to yield 10 fractions. Fractions 3–6 were decolourized on MCI gel, eluted with MeOH-H_2O (9:1). After decolourization, fraction 3 (16 g) was chromatographed over CC (250 g, 200–300 mesh) with petrol/Me_2CO (7:2), and over CC (100 g, H type) with cyclohexane-EtOAc (6:4), then cyclohexane-*i*-PrOH (12:1) (20 g, H type) to yield **1** (13 mg). Fraction 4 (20 g) was chromatographed on CC (300 g, 200–300 mesh) with petrol/Me_2CO (7:3), then over CC (110 g, H type) with cyclohexane-EtOAc (1:1) and cyclohexane-*i*-PrOH (10:1) (50 g, H type) to yield **2** (10 mg). Fraction 6 (18 g) was chromatographed on CC (330 g, 200–300 mesh) with petrol/Me_2CO (7:4), then on CC (100 g, H type) with cyclohexane-*i*-PrOH (10:1) and cyclohexane-C_6H_6-*i*-PrOH (15:3:1) (40 g, H type) to yield **3** (30 mg) and **4** (15 mg).

Compound **1**: $C_{26}H_{34}O_{10}$, white crystals, m.p. 217.5–220 °C; $[\alpha]_D^{21}$: – 1.68 (*c* 0.45, MeOH); IR: ν_{max}^{KBr} = 3350, 2930, 1710, 1630, 1430, 1360, 1260–1240 (br), 1050 cm^{-1}; UV: λ_{max}^{MeOH} (log ε) = 235.5 (3.58) nm; EIMS: *m/z* = 506 $[M]^+$, 446, 386, 344, 326, 298, 83, HRMS: *m/z* = 506.2157, required: 506.2152, ^{1}H-NMR ($CDCl_3$): δ = 6.02 (1H, brs, H-17a), 5.80 (1H, brd, *J* = 11.3 Hz, D_2O exchangeable, OH-6β), 5.50 (1H, brs, H-17b), 4.84 (1H, m,

H-11β), 4.71 (1H, dd, J = 11.4, 4.8 Hz, H-1β), 4.48 (1H, d, J = 10.9 Hz, H-19a), 4.43 (1H, d, J = 9.4 Hz, H-20a), 4.10 (2H, overlapped, H-19a and H-20a), 3.96 (1H, brt, J = 11.3 Hz, collapsed to a d after addition of D_2O, H-6α), 3.04 (1H, m, H-13α), 2.78 (1H, d, J = 12.3 Hz, H-14α), 2.35 (1H, dd, J = 15.9, 9.1 Hz, H-12α), 2.14, 2.05, 1.88 (each 3H, s, 3 × OAc), 2.10 (1H, m, H-14β), 1.74 (1H, m, H-3α), 1.71 (1H, m, H-2α), 1.67 (1H, m, H-12α), 1.62 (1H, d, J = 3.6 Hz, H-9β), 1.51 (1H, d, J = 11.3 Hz, H-5β), 1.32 (1H, m, H-2β), 1.25 (3H, s, Me-18), 1.14 (1H, m, H-3β); The ^{13}C-NMR data see Table **1**.

Compound **2**: $C_{22}H_{30}O_7$, white crystals, m.p. 187 – 189.5 °C; $[\alpha]_D^{23}$: − 40.50 (*c* 0.50, MeOH); IR: ν_{max}^{KBr} = 3400, 2940, 2910, 2840, 1715, 1650, 1450, 1360, 1240, 1110, 1050 cm^{-1}; EIMS: *m/z* = 406 $[M]^+$, 388, 328, 300, 282, 161, 55, HRMS: *m/z* = 406.2009, required: 406.1992; ^{1}H-NMR ($CDCl_3$: CD_3OD, 10 : 1): δ = 5.16 (1H, brs, H-17a), 5.11 (2H, m, H-11β and H-17b), 4.87 (1H, brs, H-15β), 4.71 (1H, brs, H-6α), 4.50 (1H, dd, J = 11.3, 6.2 Hz, H-1β), 3.84 (1H, d, J = 9.2 Hz, H-20a), 3.76 (1H, d, J = 9.2 Hz, H-20b), 2.90 (1H, d, J = 10.4 Hz, H-9α), 2.73 (1H, m, H-12β), 2.69 (1H, m, H-13β), 1.91 (3H, s, OAc), 1.84 (2H, m, H-2α and H-14β), 1.68 (1H, m, H-2β), 1.52 (1H, m, H-14α), 1.40 (1H, m, H-3α), 1.33 (1H, m, H-12α), 1.14 (1H, m, H-3β), 0.86 (3H, s, Me-19), 0.84 (3H, s, Me-18). The ^{13}C-NMR data see Table**1**.

Compound **3**: $C_{26}H_{38}O_9$, white needle crystals, m.p. 212.5 – 215 °C; $[\alpha]_D^{21}$: − 15.04 (*c* 0.27, MeOH); IR: ν_{max}^{KBr} = 3340 – 3240 (br), 2940 – 2900 (br), 1710, 1360, 1250, 1040 cm^{-1}; EIMS: *m/z* = 494 $[M]^+$, 448, 388, 360, 346, 328, 310, 282, 55, HRMS: *m/z* = 494.2566 required: 494.2516; ^{1}H-NMR (pyridine-d_5): δ = 6.02 (1H, d, J = 12.0 Hz, OH-6β), 5.19 (1H, t, J = 3.9 Hz, H-11β), 5.08 (1H, dd, J = 11.6, 5.1 Hz, H-1β), 4.72 (1H, d, J = 9.1 Hz, H-20a), 4.33 (1H, dd, J = 12.0, 8.0 Hz, H-6α), 4.26 (1H, d, J = 9.1 Hz, H-20b), 3.65 (1H, dd, J = 10.0, 4.2 Hz, H-17a), 3.55 (1H, m, H-17b), 3.25 (2H, q, J = 7.0 Hz, -OCH_2CH_3), 3.22 (1H, d, J 12.4 Hz, H-14α), 2.90 (1H, m, H-16α), 2.65 (1H, m, H-13α), 2.54 (1H, dd, J = 12.4, 3.9 Hz, H-14β), 2.18 (1H, overlapped, H-12α), 2.15 (1H, overlapped, H-9β), 2.14, 2.07 (each 3H, s, 2 × OAc), 2.00 (1H, m, H-12β), 1.76 (1H, m, H-2α), 1.62 (1H, d, J = 8.0 Hz, H-5β), 1.42 (1H, m, H-2β), 1.29 (2H, m, H_2-3), 1.26 (3H, s, Me-18), 1.09 (3H, s, Me-19), 1.03 (3H, t, J = 7.0 Hz, -OCH_2CH_3). The ^{13}C-NMR data see Table **1**.

Compound **4**: $C_{26}H_{38}O_9$, white crystals, m.p. 224 – 225.5 °C; $[\alpha]_D^{22}$: 5.68° (*c* 0.26, MeOH); IR: ν_{max}^{KBr} = 3360, 2920, 2860, 1715, 1360, 1250, 1050 cm^{-1}; EIMS: *m/z* = 494 $[M]^+$, 448, 388, 360, 346, 328, 310, 282, 55; HRMS: 494.2523 required: 494.2516; ^{1}H-NMR ($CDCl_3$): δ = 5.45 (1H, d, J = 12.0 Hz, OH-6β), 4.86 (1H, t, J = 4.0 Hz, H-11β), 4.70 (1H, m, H-1β), 4.40 (1H, d, J = 8.9 Hz, H-20a), 4.07 (1H, d, J = 8.9 Hz, H-20b), 3.92 (1H, dd, J = 12.0, 8.2 Hz, H-6α), 3.44 (3H, overlapped, H-17a and -OCH_2CH_3), 3.35 (1H, m, H-17b), 2.70 (1H, d, J = 11.3 Hz, H-14α), 2.52 (1H, brd, J = 6.7 Hz, H-13α), 2.36 (2H, overlapped, H-12α and H-16β), 2.22 (1H, brd, J = 11.2 Hz, H-14β), 2.09, 1.90 (each 3H, s, 2 × OAc), 1.73 (1H, brd, J = 9.3 Hz, H-3α), 1.63 (1H, brdd, J = 15.9, 4.0 Hz, H-12β), 1.51 (1H, brs, H-9β), 1.42 (1H, m, H-2α), 1.33 (1H, overlapperd, H-5β), 1.27 (1H, m, H-2β), 1.18 (3H, s, Me-18), 1.15 (6H, overlapped, Me-19 and -OCH_2CH_3). The ^{13}C-NMR data see Table **1**.

Copies of the original spectra are obtainable from the author of correspondence.

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