

## Four New Diterpenoids from *Isodon leucophylla*

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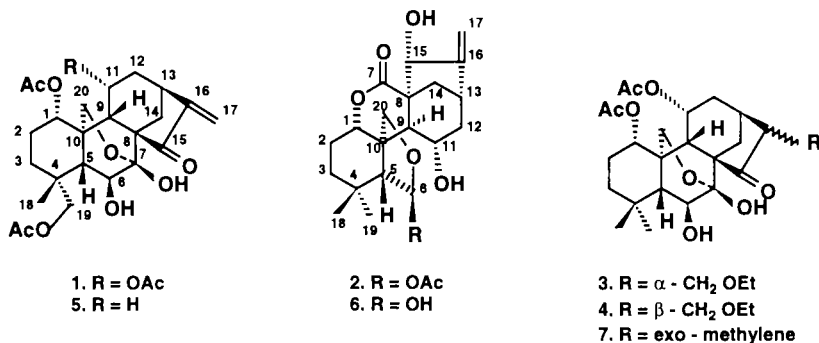
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**Abstract:** Four new diterpenoids, baiyecrystals **A-C** and *epi*-baiyecrystal **C**, were isolated from *I. leucophylla*. Their structures were determined by means of spectral methods as **1-4**.

**Keywords:** *Isodon leucophylla*; *labiatae*; *ent*-kaurane diterpenoids; baiyecrystal **A-C**; *epi*-baiyecrystal **C**.



*Isodon leucophylla* (Dunn) Hara (Labiatae), distributed in the northwest area of Yunnan Province, is used as folk medicine for its antibacterial and anti-inflammatory activity by local practitioners<sup>1</sup>. From the leaves of this plant, four new *ent*-kaurane type diterpenoids, baiyecrystal **A-C** **1-3** and *epi*-baiyecrystal **C** **4**, were isolated. This paper presents their structure elucidation.

Baiyecrystal **A** **1** C<sub>26</sub>H<sub>34</sub>O<sub>10</sub> was determined by HRMS (506.2157, calc 506.2152). Its mass spectrum showed that the molecular ion (*m/z* 506) was 58 amu more than that of effusanin **D** **5**<sup>2</sup>, a known kauranoid also isolated from this plant. The <sup>1</sup>H, <sup>13</sup>C and DEPT spectra of **1** were very similar to those of **5**. The only difference between **1** and **5** was

that **1** had one more acetoxy group. Inspection of  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of **1**, there was following partial structure:  $-\text{CHCHCH}_2\text{CHCH}_2-$  different that in **5**. The COLOC spectrum of **1** exhibited the methine signal at 4.84 ppm correlation with a quaternary carbon signal at 58.0 ppm (C-8), a methine carbon signal at 33.4 ppm (C-13) and an acetyl group 169.7 ppm. Thus, the methine should be assigned to C-11 which attached an acetoxy group. When C-7 and C-20 formed an epoxy, the C-ring changed from chair-conformation to boat-conformation. The relative configuration of C-11-OAc was assigned to  $\alpha$ -orientation on the basis of the coupling constant of H-9 $\beta$  with H-11 $\alpha$  ( $J=3.6$  Hz) and the broad multiplet signal at 4.84 ppm for H-11. Therefore, the structure of **1** should be  $1\alpha$ ,  $11\alpha$ ,  $19$ -triacetoxy- $6\beta$ ,  $7\beta$ -dihydroxyl- $7$ ,  $20$ -epoxy-*ent*-kaur- $16$ -en- $15$ -one.

Baiyecrystal **B 2** was determined as  $\text{C}_{22}\text{H}_{30}\text{O}_7$  by HRMS (406.2009, calc 406.1992), mp 187.0-189.5 $^\circ$ , EIMS (70eV)  $m/z$  (rel. Int %): 406[M] $^+$  (10), 388 (25), 328 (30), 300 (32), 282 (47), 161 (75), 55 (100). Its mass spectrum showed that molecular ion ( $m/z$  406) was 42 amu more than that of epinodosinol **6**<sup>3-5</sup>. Its  $^1\text{H}$ ,  $^{13}\text{C}$  and DEPT spectra were very similar to those of **6**. The only difference between **2** and **6** was that **2** had one more acetoxy group and the chemical shift value of a methine carbon in **2** was downshifted about 7 ppm. It is very difficult to assign the place of substituent at C-6 or C-20 when the usual spectroscopic methods are employed. Inspection of  $^1\text{H}$ - $^1\text{H}$  and  $^{13}\text{C}$ - $^1\text{H}$  COSY spectra of **2**, downfield methylene ( $\delta$  72.7) were observed as two doublet signals without any vicinal correlation. Thus, the methylene should be assigned to C-20. And the downfield methine ( $\delta$  109.3) should be assigned to C-6. Comparison the  $^{13}\text{C}$  NMR data of **6** with those of **2**, the chemical shift value of C-6 changed from 102.2 ppm in **6** to 109.3 ppm in **2** and the chemical shift value of C-11 changed from 63.0 ppm in **6** to 66.8 ppm in **2**. Consideration of the solvent effect, the acetoxy group should be attached to C-6 which was further confirmed by COLOC experiment, i.e. there was a cross-peak between H-6 (4.71 ppm) and acetyl group ( $\delta$  170.9 ppm) in the COLOC spectrum. The relative configuration of C-6-OAc was  $\beta$ -orientation on the basis of a broad singlet signal at 4.71 ppm in  $^1\text{H}$  NMR spectrum because of the near  $90^\circ$  dihedral angle with H-5. The relative configuration of the other substituents were indicated by coupling constants of H-1 $\beta$  with H-2 $\alpha$  ( $J=11.2$  Hz) and H-2 $\beta$  ( $J=6.2$  Hz); H-9 $\alpha$  with H-11 $\beta$  ( $J=10.4$  Hz) and the multiplet at 5.13 ppm for H-11, whereas, H-15 $\beta$  was assigned to  $\beta$ -orientation on the basis of comparison of the  $^{13}\text{C}$  data and  $^1\text{H}$  data with **6**. Therefore, the structure of **2** should be  $6\beta$ -acetoxy- $11\alpha$ ,  $15\alpha$ -dihydroxyl- $6$ ,  $20$ -epoxy- $6,7$ -*seco-ent*-kaur- $16$ -en- $1,7$ -olide.

Baiyecrystal **C 3**  $\text{C}_{26}\text{H}_{38}\text{O}_9$  (HRMS 494.2566, calc 494.2516), mp 212.5-215 $^\circ$ ,  $[\alpha]_D^{21.2}$  -15.04 (MeOH, c 0.27), EIMS (70eV)  $m/z$  (rel. Int %): 494[M] $^+$  (20), 448 (70), 388 (50), 360 (15), 346 (25), 328 (50), 310 (65), 282 (50), 55 (100). The  $^1\text{H}$ ,  $^{13}\text{C}$  and DEPT spectra of **3** showed the presence of two methyls (C-18 and C-19), six methylenes (C-2, C-3, C-12, C-14, C-17 and C-20), four quaternary carbons (C-4, C-7, C-8 and C-10), seven methines (C-1, C-5, C-6, C-9, C-11, C-13 and C-16), a ketonic carbon (C-15), two acetoxy functions (attached to C-1 and C-11), and an ethoxyl group [66.5 ( $t$ ) and 15.2 ( $q$ )] in  $^{13}\text{C}$  NMR, and 3.25 (2H,  $q$ ,  $J=7.0$  Hz), 1.03 (3H,  $t$ ,  $J=7.0$  Hz) in  $^1\text{H}$  NMR]. These signals indicated that **3** was a type epoxy-*ent*-kauranoid with four substituents besides the ethoxyl signals. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of **3** were very similar to those of shikokianin (**7**)<sup>6</sup>, a known kaurenoid also isolated from this plant, except for the D-ring.

Instead of the *exo*-methylene signals in **7**, signals for a methine (56.1 ppm) and methylene bearing oxygen (67.1 ppm) carbons were observed in **3**. In the  $^{13}\text{C}$ - $^1\text{H}$  COSY and  $^1\text{H}$ - $^1\text{H}$  COSY spectra of **3**, the methine (56.1 ppm) signal at  $\delta$  2.90 revealed correlation with another methine (29.1 ppm, C-13) signal at  $\delta$  2.65 and the methylene (67.1 ppm) signals at  $\delta$  3.65 and 3.55. Thus, the methine and methylene should be assigned to C-16 and C-17 and its bearing oxygen should be assigned to an ethoxyl group. The relative configuration of ethoxymethyl group was assigned to  $\beta$ -orientation deduced from the unusual upfield (from 37.7 ppm in **7** to 28.9 ppm in **3**) of C-12<sup>7,8</sup> cause by the  $\gamma$ -effect of the ethoxymethyl. Therefore, **3** was determined as  $1\alpha$ ,  $11\alpha$ -diacetoxy- $6\beta$ ,  $7\beta$ -dihydroxy- $16\beta$ -ethoxymethyl-*ent*-7,20-epoxy-kaur-15-one.

*epi*-Baiyecrystal **C 4**  $\text{C}_{26}\text{H}_{38}\text{O}_6$  (HRMS 494.2523, calc 494.2516); mp 224-225.5°;  $[\alpha]_{\text{D}}^{22.3}$  -5.68 (MeOH,  $c$  0.26); EIMS (70 eV)  $m/z$  (rel. int %): 494 [M]<sup>+</sup> (15), 448 (60), 388 (40), 360 (10), 346 (15), 328 (60), 310 (60), 282 (45), 55 (100). It has the same molecular ion as that of Baiyecrystal **C 3**. The  $^1\text{H}$ ,  $^{13}\text{C}$  and DEPT spectra of **4** were very similar to those of **3**. The  $^{13}\text{C}$ ,  $^{13}\text{C}$ - $^1\text{H}$  COSY and COLOC spectra of **4** and those of **3**, indicated that the only difference between **4** and **3** was that the C-12 chemical shift value changed from 28.9 ppm in **3** to 37.9 ppm in **4**, and the C-14 chemical shifted value changed from 28.6 ppm in **3** to 25.82 ppm in **4**. Another evidence was that the  $^{13}\text{C}$  NMR data of C-17 ( $\delta$  67.1) in **3** shifted downfield for about 2.6 ppm ( $\delta$  69.7) in **4**. The above evidences indicated that **4** was the epimer of **3**. Therefore, the structure of **4** was deduced as  $1\alpha$ ,  $11\alpha$ -diacetoxy- $6\beta$ ,  $7\beta$ -dihydroxy- $16\alpha$ -ethoxymethyl-*ent*-7, 20-epoxy-kaur-15-one.

The two compounds may be artifacts from shikokianin **7**, but we have not any evidence to confirm them.

The list of the NMR data of baiyecrystal **A** and *epi*-baiyecrystal **C**:

Baiyecrystal **A**  $^1\text{H}$  NMR  $\delta$ ( $\text{CDCl}_3$ ): 6.02(1H, brs, H-17a), 5.80(1H, brd,  $J = 11.3$  Hz,  $\text{D}_2\text{O}$  exchangeable, OH-6 $\beta$ ), 5.50(1H, brs, H-17b), 4.84(1H, m, H-11 $\beta$ ), 4.71(1H, dd,  $J = 11.4, 4.8$  Hz, H-1 $\beta$ ), 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,  $\text{D}_2\text{O}$  exchanged to d, H-6 $\alpha$ ), 3.04(1H, m, H-13 $\alpha$ ), 2.14, 2.05, 1.88(each 3H, s,  $3\times\text{OAc}$ ), 1.62(1H, d,  $J = 3.6$  Hz, H-9 $\beta$ ), 1.25(3H, s, Me-18).  $^{13}\text{C}$  NMR  $\delta$ : 75.8 (d) (C1), 24.6 (t) (C2), 32.8 (t) (C3), 36.3 (s) (C4), 58.3 (d) (C5), 73.3 (d) (C6), 94.9 (s) (C7), 58.0 (s) (C8), 53.1 (d) (C9), 41.3 (s) (C10), 69.3 (d) (C11), 37.7 (t) (C12), 33.4 (d) (C13), 25.7 (t) (C14), 207.2 (s) (C15), 151.3 (s) (C16), 119.6 (t) (C17), 28.7 (q) (C18), 66.8 (t) (C19), 65.1 (t) (C20);  $3\times\text{OAc}$ : 170.9, 170.8, 169.7; 22.0, 21.5, 20.9.

Baiyecrystal **B**  $^1\text{H}$  NMR  $\delta$  ( $\text{CDCl}_3$ - $\text{CD}_3\text{OD}=10:1$ ): 5.16 (1H, brs, H-17a), 5.11 (2H, overlapped, H-11 $\beta$  and H-17b), 4.87 (1H, brs, H-15 $\beta$ ), 4.71 (1H, brs, H-6 $\alpha$ ), 4.50(1H, dd,  $J = 11.3, 6.2$  Hz, H-1 $\beta$ ), 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-9 $\alpha$ ), 2.69(1H, m, H-13 $\beta$ ), 1.91(3H, s, OAc), 0.86(3H, s, Me-19), 0.84(3H, s, Me-18).  $^{13}\text{C}$  NMR  $\delta$ : 76.5(d)(C1), 23.2(t)(C2), 36.2(t)(C3), 30.8 (s)(C4), 52.7(d)(C5), 109.3(d)(C6), 175.2(s)(C7), 52.9(s)(C8), 41.9(d)(C9), 50.6(s)(C10), 66.8(d)(C11), 41.0(t)(C12), 35.9(d)(C13), 34.3(t) (C14), 77.2(d)(C15), 155.2(s)(C16), 109.3(t)(C17), 32.4(q)(C18), 23.5(q)(C19), 72.7(t) (C20); OAc: 170.9, 21.1.

Baiyecrystal **C**  $^1\text{H}$  NMR  $\delta$ (pyridine- $d_5$ ): 6.02(1H, d,  $J = 12.0$  Hz, OH-6 $\beta$ ), 5.19(1H, t,  $J = 3.9$  Hz, H-11 $\beta$ ), 5.08 (1H, dd,  $J = 11.6, 5.1$  Hz, H-1 $\beta$ ), 4.72(1H, d,  $J = 9.1$  Hz, H-

20a), 4.33(1H, *dd*,  $J = 12.0, 8.0$  Hz, H-6 $\alpha$ ), 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<sub>2</sub>CH<sub>3</sub>), 2.90(1H, *m*, H-16 $\alpha$ ), 2.65(1H, *m*, H-13 $\alpha$ ), 2.14, 2.07(each 3H, *s*, 2 $\times$ OAc), 1.26(3H, *s*, Me-18), 1.09(3H, *s*, Me-19), 1.03(3H, *t*,  $J = 7.0$  Hz, -OCH<sub>2</sub>CH<sub>3</sub>). <sup>13</sup>C NMR  $\delta$ : 76.6(*d*) (C1), 25.4(*t*) (C2), 39.0(*t*) (C3), 33.7(*s*) (C4), 58.9(*d*) (C5), 75.1(*d*) (C6), 95.8(*s*) (C7), 59.7(*s*) (C8), 51.6(*d*) (C9), 41.4(*s*) (C10), 70.2(*d*) (C11), 28.9(*t*) (C12), 29.0(*d*) (C13), 28.6(*t*) (C14), 221.2(*s*) (C15), 56.1(*d*) (C16), 67.1(*t*) (C17), 34.1(*q*) (C18), 22.7(*q*) (C19), 64.9(*t*) (C20); 2 $\times$ OAc: 170.5, 170.1, 22.0, 21.8; OCH<sub>2</sub>CH<sub>3</sub>: 66.5(*t*), 15.2(*q*).

*epi*-Baiyecrystal C <sup>1</sup>H NMR  $\delta$ (CDCl<sub>3</sub>): 5.45(1H, *d*,  $J = 12.0$  Hz, OH-6 $\beta$ ), 4.86(1H, *t*,  $J = 4.0$  Hz, H-11 $\beta$ ), 4.70(1H, *m*, H-1 $\beta$ ), 4.40(1H, *d*,  $J = 8.8$  Hz, H-20a), 4.07(1H, *d*,  $J = 9.1$  Hz, H-20b), 3.92(1H, *dd*,  $J = 12.0, 8.2$  Hz, H-6 $\alpha$ ), 3.44(3H, overlapped, H-17a and -OCH<sub>2</sub>CH<sub>3</sub>), 3.35(1H, *m*, H-17b), 2.52(1H, *brd*,  $J = 6.7$  Hz, H-13 $\alpha$ ), 2.36(2H, overlapped, H-12 $\alpha$  and H-16 $\beta$ ), 2.09, 1.90(each 3H, *s*, 2 $\times$ OAc), 1.18(3H, *s*, Me-18), 1.15(6H, overlapped, Me-19 and -OCH<sub>2</sub>CH<sub>3</sub>). <sup>13</sup>C NMR  $\delta$ : 76.4 (*d*) (C1), 25.0 (*t*) (C2), 38.8 (*t*) (C3), 33.4 (*s*) (C4), 57.6 (*d*) (C5), 74.7 (*d*) (C6), 94.6 (*s*) (C7), 58.3 (*s*) (C8), 53.3 (*d*) (C9), 41.4 (*s*) (C10), 69.4 (*d*) (C11), 37.9 (*t*) (C12), 29.4 (*d*) (C13), 25.8 (*t*) (C14), 220.0 (*s*) (C15), 57.8 (*d*) (C16), 69.7 (*t*) (C17), 33.8 (*q*) (C18), 22.8 (*q*) (C19), 64.9 (*t*) (C20), 2 $\times$ OAc: 170.3, 169.7, 22.0, 21.6; OCH<sub>2</sub>CH<sub>3</sub>: 66.7 (*t*), 15.0 (*q*).

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