

## Four New Compounds From *Isodon eriocalyx*

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**Abstract** – Examination of the chemical constituents of the dried leaves of *Isodon eriocalyx* (Dunn.) Hara led to the isolation of four new diterpenoids, named maoecrystal Q-T (1-4). Their structures were elucidated as 16(R)-methoxymethyl-6 $\beta$ , 7 $\beta$ -dihydroxy-7 $\alpha$ ,20-epoxy-*ent*-kaur-2,3-ethenylene-1, 15-dione(1), 1 $\beta$ ,18-diacetoxy-6 $\beta$ ,7 $\beta$ -dihydroxy-7 $\alpha$ ,20-epoxy-*ent*-kaur-16-en-15-one (2), 6 $\beta$ , 7 $\beta$ -dihydroxy-15 $\beta$ -acetoxy-7 $\alpha$ ,20-epoxy-*ent*-kaur-16-en-1-one (3) and 7 $\beta$ -hydroxy-6 $\beta$ ,15 $\beta$ -diacetoxy-1 $\alpha$ ,11 $\beta$ -acetone-7 $\alpha$ , 20-epoxy-*ent* -kaur-16-ene (4), respectively, on the basis of spectroscopic methods. Meanwhile, five known diterpenoids eriocalyxin B (5), maoecrystal B-D (6-8) and trichokaurin (9) were also obtained.

**Key words** – *Isodon eriocalyx*, Labiatae, diterpenoids.

### Introduction

Plants belonging to the *Isodon* genus are known to be rich in *ent*-kaurane diterpenoids, a number of which possess various biological activities such as cytotoxic (Sun *et al.*, 1995), antitumor (Fuji *et al.*, 1989) and antibacterial activities (Fujita *et al.*, 1976).

As a part of our search for biologically active constituents of this genus, the present paper deals with the isolation and identification of four new diterpenoids named maoecrystal Q-T (1-4) along with five known compounds eriocalyxin B (5) (Wang *et al.*, 1982), maoecrystal B-D (6-8) (Li *et al.*, 1988) and trichokaurin (9) (Fujita *et al.*, 1967) from *I. eriocalyx* (Dunn.) Hara.

### Experimental

#### General experimental procedures –

Melting points were uncorrected. Optical rotations were taken on a JASCO-20C digital polarimeter. IR spectra were recorded with a Perkin-Elmer 577 spectrometer. UV spectra were obtained on a UV 210A spectrometer. MS spectra were measured on a VG Autospec-3000 spectrometer. NMR spectra were run on a Bruker AM- 400 spectrometer. The chemical shifts ( $\delta$ ) were expressed in ppm with reference to the solvent signals. Coupling constants (J) were given in Hz.

**Plant material** – The dried leaves of *Isodon eriocalyx* (Dunn.) Hara were collected in Jiangchuan County, Yunnan Province, China, in 1994 and identified by Prof. Xi-Wen Li. A voucher specimen is deposited in the Herbarium of Kunming Institute of Botany, Academia Sinica, Kunming, Yunnan, P. R. China.

**Extraction and isolation** – Dried powdered leaves of *I. eriocalyx* (Dunn.) Hara


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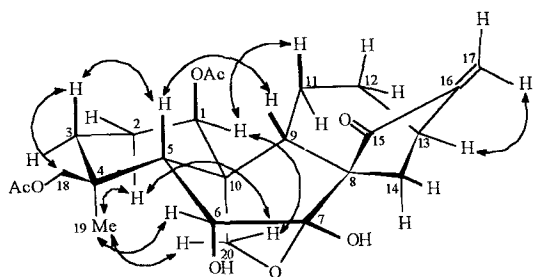
(11.9 kg) were extracted with MeOH (4×40l) under reflux and then concentrated in vacuum to give crude extract (978 g). The extract was dissolved in H<sub>2</sub>O and successively extracted with petroleum ether (60-90°C) and ethyl acetate. The AcOEt Part (395 g) was chromatographed on a silica gel column (1.6 kg, 200-300 mesh) eluting with CHCl<sub>3</sub> by increasing amount of Me<sub>2</sub>CO to yield seven fractions. Fraction I, II and III were submitted to MCI gel to decolor green pigments. Then each fraction was subjected to silica gel column chromatography repeatedly followed by recrystallization and finally afforded nine compounds: **1** (1.1 g), **2** (200 mg), **3** (100 mg), **4** (30 mg), **5** (4.1 g), **6** (1.1 g), **7** (1.2 g), **8** (197 mg) and **9** (15 mg).

Maoecrystal Q (**1**). C<sub>21</sub>H<sub>28</sub>O<sub>8</sub>, colorless crystals (from MeOH), mp: 180.0-182.5°C,  $[\alpha]_D^{25}$

-117.5° (CHCl<sub>3</sub>, c 0.283); IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>-1</sup>: 3250, 2940, 1710, 1656, 1485, 1370, 1300, 1265, 1210, 1160, 1104 and 1050; UV  $\lambda_{\max}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 227 (3.83); EIMS (70 eV) m/z (rel. int. %): 376 [M]<sup>+</sup> (83), 358 [M-H<sub>2</sub>O]<sup>+</sup> (34), 348 (66), 329 (49), 317 (84), 298 (33), 255 (35), 227 (31), 193 (32), 165 (100), 149 (33), 135 (48), 105 (47), 96 (84) and 79 (55); HR EIMS m/z: found: 376.1864 [M]<sup>+</sup> (calc. 376.1886); <sup>1</sup>H NMR (pyridine-d<sub>5</sub>)  $\delta$ : 5.97 (1H, d,  $J=10.1$  Hz, H-2), 6.67 (1H, d,  $J=10.1$  Hz, H-3), 4.26 (1H, d,  $J=7.6, 11.6$  Hz, H-6 $\alpha$ ), 1.98 (1H, dd,  $J=4.8, 13.6$  Hz, H-9 $\beta$ ), 2.64 (1H, m, H-13 $\alpha$ ), 2.89 (1H, m, H-16 $\alpha$ ), 3.63 (1H, d,  $J=10.2$  Hz, H-17b), 3.67 (1H, d,  $J=10.2$  Hz, H-17a), 1.34 (3H, s, CH<sub>3</sub>-18), 1.07 (3H, s, CH<sub>3</sub>-19), 4.11 (1H, dd,  $J=1.2, 10.1$  Hz, H-20b), 4.52 (1H, d,  $J=10.1$  Hz, H-20a), 6.14 (1H, d,  $J=11.6$  Hz, OH-6 $\beta$ ) and 3.21 (3H, s, OCH<sub>3</sub>); <sup>13</sup>C NMR data see Table 1.

**Table 1.** <sup>13</sup>C NMR data of **1-4**, **5** and **8** in C<sub>5</sub>D<sub>5</sub>N (100 MHz,  $\delta$  in ppm with reference to the signal of C<sub>5</sub>D<sub>5</sub>N)

Carbon	<b>1</b>	<b>2</b>	<b>5</b>	<b>3</b>	<b>4</b>	<b>8</b>
1	197.3	72.8	197.3	213.3	75.1	212.2
2	127.4	22.7	127.4	36.0	27.8	35.7
3	160.6	23.2	106.9	39.2	40.5	38.6
4	36.2	36.3	36.1	32.9	33.6	32.8
5	59.3	57.4	58.5	56.2	54.2	54.3
6	73.6	73.3	73.6	73.8	73.8	74.1
7	96.3	96.1	96.7	97.6	95.4	96.3
8	61.1	60.2	60.4	52.2	51.7	52.2
9	48.1	50.0	48.4	42.9	50.2	42.8
10	46.3	41.4	46.7	49.3	39.6	49.1
11	19.3	16.8	19.6	18.0	63.7	17.9
12	20.0	29.5	30.0	32.5	40.1	32.3
13	29.7	35.1	34.8	36.1	37.0	36.0
14	28.1	26.7	25.9	27.2	26.5	27.1
15	222.5	210.2	209.2	75.2	74.8	75.3
16	56.6	153.7	153.7	159.4	159.1	158.7
17	69.0	116.6	117.7	109.1	109.4	109.7
18	30.3	66.8	30.2	31.2	32.5	29.5
19	24.2	21.8	24.5	23.7	22.7	23.0
20	65.6	66.4	65.5	64.9	64.6	64.9
OAc		170.7		170.8	171.1	170.8
		170.3			171.1	170.6
		20.9		22.1	21.9	22.0
		20.5			21.2	21.2
OCH <sub>3</sub>	58.6					
					100.9	
					24.8	
					24.7	



**Fig. 1.** Expression of the major NOESY correlations in maoecrystal R.

Maoecrystal R (**2**).  $C_{24}H_{32}O_8$ , colorless crystals (from MeOH), mp: 97.5-100.0°C,  $[\alpha]_D^{25} -22.3^\circ$  ( $CHCl_3$ , c 0.382); IR  $\nu_{max}^{KBr}$   $cm^{-1}$ : 3340, 2920, 1725, 1700, 1630, 1440, 1370, 1240 and 1025; UV nm (log  $\epsilon$ ): 239.5 (3.71); EIMS (70 eV) m/z (rel. int. %): 448  $[M]^+$  (25), 430  $[M-H_2O]^+$  (4), 388 (100), 328 (41), 310 (14), 297 (27), 267 (37), 253 (37), 161 (23), 149 (44), 119 (35), 105 (58), 91 (60), 83 (72) and 55 (64); HR EIMS m/z: found: 448.2111  $[M]^+$  (calc. 448.2097);  $^1H$  NMR (pyridine- $d_5$ )  $\delta$ : 5.35 (1H, br. s, H-1 $\alpha$ ), 5.89 (1H, d,  $J=5.7$  Hz, H-5 $\beta$ ), 4.37 (1H, dd,  $J=6.4$  Hz, H-6 $\alpha$ ), 1.73-1.79 (1H, m, overlap, H-9 $\beta$ ), 2.90 (1H, dd,  $J=4.4$ , 9.5 Hz, H-13 $\alpha$ ), 2.43 (1H, d,  $J=7.9$  Hz, H-14 $\alpha$ ), 2.50 (1H, dd,  $J=7.9$ , 4.4 Hz, H-14 $\beta$ ), 5.29 (1H, br. s, H-17b), 5.96 (1H, br. s, H-17a), 4.58 (1H, d,  $J=11.5$  Hz, H-18b) 4.63 (1H, d,  $J=11.5$  Hz, H-18a), 1.43 (3H, s,  $CH_3$ -19), 4.05 (2H, br. s,  $H_2$ -20), 1.91 (3H, s, OAc) and 2.14 (3H, s, OAc);  $^{13}C$  NMR data see Table 1.

Maoecrystal S (**3**).  $C_{22}H_{30}O_6$ , colorless crystals (from MeOH), mp: 170.5-172.0°C,  $[\alpha]_D^{25} +43.5^\circ$  ( $CHCl_3$ , c 0.506), IR  $\nu_{max}^{KBr}$   $cm^{-1}$ : 3518, 3314, 2920, 1685, 1670, 1408, 1355, 1250, 1145, 1053, 1031 and 890; EIMS (70 eV) m/z (rel. int. %): 390  $[M]^+$  (29), 372  $[M-H_2O]^+$  (12), 329 (42), 287 (40), 269 (35), 228 (67), 163 (82), 145 (52), 119 (49), 105 (56), 91 (79), 69 (82) and 55 (100); HR EIMS m/z: found: 390.2084  $[M]^+$  (cacl. 390.2042);  $^1H$  NMR (pyridine- $d_5$ )  $\delta$ : 2.68 (1H, d,  $J=8.3$  Hz, H-5 $\beta$ ), 4.17 (1H, m, overlap, H-6 $\alpha$ ), 6.29 (1H, t,  $J=2.4$  Hz, H-15 $\alpha$ ), 5.13 (1H, d,  $J=2.6$  Hz, H-

17b), 5.17 (1H, br. s, H-17a), 1.22 (3H, s,  $CH_3$ -18), 0.94 (3H, s,  $CH_3$ -19), 4.17 (1H, m, overlap, H-20b), 4.64 (1H, d,  $J=9.8$  Hz, H-20a), 6.01 (1H, br. s, OH-6 $\beta$ ) and 2.31 (3H, s, OAc);  $^{13}C$  NMR data see Table 1.

Maoecrystal T (**4**).  $C_{27}H_{38}O_8$ , colorless crystals (from MeOH), mp: 237.5-239.0°C,  $[\alpha]_D^{25} -129.3^\circ$  ( $CHCl_3$ , c 0.379); IR  $\nu_{max}^{KBr}$   $cm^{-1}$ : 3370, 2922, 1725, 1645, 1363, 1238, 1214, 1160, 1068, 1040, and 885; EIMS (70 eV) m/z (rel. int. %): 490  $[M]^+$  (16), 448  $[M-ketene]^+$  (64), 430 (34), 402 (3), 388 (43), 372 (60), 330 (32), 284 (57), 255 (42), 151 (63), 105 (57), 91 (77), 69 (93) and 55 (100); HR EIMS m/z: found: 490.2591  $[M]^+$  (cacl. 490.2567);  $^1H$  NMR (pyridine- $d_5$ )  $\delta$ : 3.99 (1H, d,  $J=4.7$ , 12.4 Hz, H-1 $\beta$ ), 5.81 (1H, d,  $J=7.2$  Hz, H-6 $\alpha$ ), 4.47 (1H, m, H-11 $\alpha$ ), 6.19 (1H, t,  $J=2.2$  Hz, H-15 $\alpha$ ), 5.13 (1H, d,  $J=2.2$  Hz, H-17b), 5.25 (1H, br. s, H-17a), 1.18 (3H, s,  $CH_3$ -18), 0.91 (3H, s,  $CH_3$ -19), 4.37 (1H, d,  $J=9.9$  Hz, H-20b), 4.57 (1H, d,  $J=9.9$  Hz, H-20a), 8.46 (1H, s, OH-7 $\beta$ ), 2.10 and 2.20 (each 3H, s,  $2 \times$  OAc), 1.38 and 1.40 (each 3H, s, acetonide,  $2 \times$   $CH_3$ );  $^{13}C$  NMR data see Table 1.

## Results and Discussion

The MeOH extract of the dried leaves of *I. ericalyx* was subjected to repeated chromatography followed by recrystallization to yield maoecrystal Q-T (**1-4**).

Maoecrystal Q (**1**) was established to have a molecular formula of  $C_{21}H_{28}O_6$  by EI mass (mol. ion at m/z at 376) and  $^{13}C$  NMR (including DEPT). This was confirmed by HR EI mass (found 376.1864, calc. 376.1886).

The  $^1H$  and  $^{13}C$  NMR spectra of **1** were similar to those of ericalyxin B (**5**) except for the ring D signals. The exo-methylene at C-16 in **5** was replaced by a methoxymethyl group in **1** judging from the following spectral data:  $^1H$  NMR  $\delta$  (ppm): 2.89 (1H, m, H-16 $\alpha$ ), 3.21 (3H, s,  $OCH_3$ ), 3.63 (1H, d,  $J=10.2$  Hz, H-17b), 3.67 (1H, d,  $J=10.2$  Hz, H-

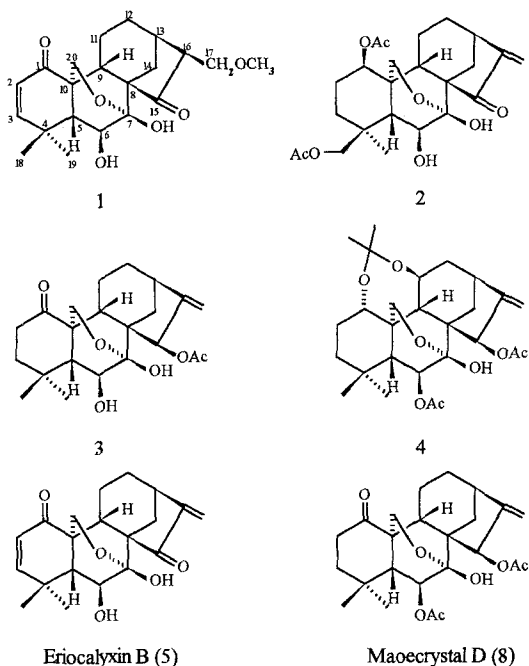


Fig. 2.

17a),  $^{13}\text{C}$  NMR  $\delta$  (ppm): 58.6 (q,  $\text{OCH}_3$ ), 56.6 (d, C-16) and 69.0 (t, C-17).

The  $\beta$ -orientation of methoxymethyl group was deduced from the upfield shift of C-12 ( $\delta$  20.0 ppm) due to the  $\gamma$ -steric compression effect between  $16\beta$ -methoxymethyl group and H-12 $\beta$ . Therefore, compound **1** was elucidated as  $16(R)$ -methoxymethyl- $6\beta,7\beta$ -dihydroxy- $7\alpha,20$ -epoxy-*ent*-kaur-2,3-ethenylene-1,15-dione.

Maoecrystal R (**2**) had a molecular formula of  $\text{C}_{24}\text{H}_{32}\text{O}_8$  deduced from EI mass (mol. ion at  $m/z$  448) and  $^{13}\text{C}$  NMR. The UV and IR absorption:  $\lambda_{\text{max}}^{\text{MeOH}}$ : 239.5 (log  $\epsilon$  3.71);  $\lambda_{\text{max}}^{\text{KBr}}$ : 1725, 1630  $\text{cm}^{-1}$  were constant with the presence of a five-membered ring with a ketone conjugated with an *exo*-methylene. The comparison of its  $^1\text{H}$  and  $^{13}\text{C}$  NMR data with those of eriocalyxin B (**5**) indicated **2** had the same skeleton like that of **5** as  $6\beta,7\beta$ -dihydroxy- $7\alpha,20$ -epoxy-*ent*-kaur-16-en-15-one and two additional acetoxy groups.

The carbon signal due to oxygen-bearing methine at  $\delta$  72.8 ppm and the downfield shift of C-10 ( $\delta$  41.4 ppm) suggested one

acetoxy group was assigned at C-1. The orientation of acetoxy could be determined by coupling constant of H-1. For  $7\alpha,20$ -epoxy-*ent*-kaurane diterpenoid, ring A is chair conformation. In this case, H-1 $\beta$  is axial and the coupling constants between H-1 $\beta$  with H-2 $\alpha$  and H-2 $\beta$  are  $J_{1\beta,2\alpha}=8-12$  Hz,  $J_{1\beta,2\beta}=4-6$  Hz, respectively, while H-1 $\alpha$  is equatorial and the dihedral angles between H-1 $\alpha$  with H-2 $\alpha$  and H-2 $\beta$  are both about  $60^\circ$ , thus the coupling constant is as small as 3 Hz or so. Because of this, the peak form of H-1 $\alpha$  signal is usually triplet with coupling constant as about 3 Hz, or even broad singlet. The signal ascribable to H-1 was broad singlet, thus acetoxy at C-1 was  $\beta$ -orientation.

Another acetoxy was determined at C-18 because only one methyl signal assignable to C-19 at  $\delta$  21.8 ppm was observed. The oxygen-bearing methylene signal at  $\delta$  66.8 ppm confirmed this. Furthermore, the upfield shift of C-3 ( $\delta$  23.2 ppm) could only be explained by the  $\gamma$ -steric compression effect of  $1\beta$ -acetoxy and  $18$ -acetoxy-methylene together. Only one group couldn't result in such big upfield shift at C-3.

The structure of **2** was supported by NOESY spectrum. In the NOESY spectrum of **2**, NOE cross peaks were observed for (i) H-1 $\alpha$  and H-11 $\beta$ , H-20a, (ii) H-5 $\beta$  and H-3 $\beta$ , H-9 $\beta$ , (iii) H-2 $\alpha$  and  $\text{CH}_3$ -19, H-20a, (iv)  $\text{CH}_3$ -19 and H-6 $\alpha$ , H-20b (v) H-17b and H-13 $\alpha$  (vi) H-3 $\beta$  and H-18b.

Thus, compound **2** was determined as  $1\beta,18$ -diacetoxy- $6\beta,7\beta$ -dihydroxy- $7\alpha,20$ -epoxy-*ent*-kaur-16-en-15-one.

Maoecrystal S (**3**) had a molecular formula of  $\text{C}_{22}\text{H}_{30}\text{O}_6$  inferred from the analysis of EI mass (mol. ion at  $m/z$  390) and  $^{13}\text{C}$  NMR as well as DEPT spectra.

The comparison of  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra in **3** with those of maoecrystal D (**8**) showed that **3** was a deacetyl derivative of **8**. The upfield shift of H-6 signal from  $\delta$  5.67 in **8** to  $\delta$  4.17 ppm in **3** suggested that **3** was 6-

deacetyl derivative of **8**. This was also confirmed by the upfield shift of C-6 signal ( $-0.3$  ppm) and downfield shift of C-5 ( $+1.9$  ppm) and C-7 ( $+1.3$  ppm), respectively.

The  $\alpha$ -orientation of H-6 was inferred from the coupling constant between H-5 $\beta$  and H-6 ( $J=8.3$  Hz). Finally, compound **3** was determined as 6 $\beta$ , 7 $\beta$ -dihydroxy-15 $\beta$ -acetoxy-7 $\alpha$ ,20-epoxy-*ent*-kaur-16-en-1-one.

Maoecrystal T (**4**) was obtained as colorless crystals (from MeOH). The molecular formula  $C_{27}H_{38}O_8$  was analyzed from EI mass (mol. ion at  $m/z$  490) and  $^{13}C$  NMR as well as DEPT spectra. The comparison of its  $^1H$  and  $^{13}C$  NMR data with those of maoecrystal D (**8**) indicated that **4** had the skeleton of 7 $\beta$ -hydroxy-6 $\beta$ , 15 $\beta$ -diacetoxy-7 $\alpha$ ,20-epoxy-*ent*-kaur-16-ene. The signals at  $\delta_c$  100.9 (s), 24.7 (q), 24.8 (q) ppm and  $\delta_H$  1.38, 1.40 ppm (each 3H, s) proved the presence of acetonide group. In *ent*-kaurane diterpenoids, the oxygen-bearing methine carbon atoms of which chemical shifts are below 70.0 ppm should be located at C-2 or C-11 (including C-6 only when C-7 was non-oxygenated) (Qing *et al.*, 1984). Thus, the carbon signal at  $\delta$  63.7 (d) ppm and the downfield shifts of C-9 (50.2 ppm) and C-12 (40.1 ppm) suggested C-11 was oxygenated.

Another carbon signal at  $\delta$  75.1 (d) ppm assigned to C-1 was supported by the downfield shifts of C-10 ( $\delta$  39.6 ppm) and C-2 ( $\delta$  27.8 ppm), respectively. Meanwhile, the proton signals at  $\delta$  3.99 (1H, d,  $J=4.7$ , 12.4 Hz, H-1 $\beta$ ) and  $\delta$  4.47 ppm (1H, m, H-11 $\alpha$ ) demonstrated the existence of 1 $\alpha$ ,11 $\beta$ -acetonide.

From all the evidence mentioned above, compound **4** was elucidated as 7 $\beta$ -hydroxy-6 $\beta$ ,15 $\beta$ -diacetoxy-1 $\alpha$ ,11 $\beta$ -acetonide-7 $\alpha$ ,20-

epoxy-*ent*-kaur-16-ene. It appeared to be an artifact.

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