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# *ENT*-KAURENE DITERPENOIDS FROM *RABDOSIA ERIOCALYX*

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**Key Word Index**—*Rabdosia eriocalyx*; Labiatae; *ent*-kaurene; diterpenoid; maoecrystal I; maoecrystal J; plant growth inhibitor.

**Abstract**—Two new *ent*-kaurene diterpenoids, maoecrystal I and maoecrystal J, were isolated from the leaves of *Rabdosia eriocalyx*. Their structures were determined by detailed spectroscopic analyses. Both maoecrystal I and J inhibited root growth of lettuce seedlings.

## INTRODUCTION

*Rabdosia eriocalyx* Hara is widely distributed in Yunnan, China. It is used in folk medicine to reduce swellings. The leaves of *R. eriocalyx* Hara collected in different areas in Yunnan, have yielded a series of new *ent*-kaurene diterpenoid derivatives [1, 2]. As part of our search for biologically active constituents of that plant, the present paper deals with the isolation and the structure elucidation of two new *ent*-kaurene diterpenoids which we have named maoecrystal I (**1**) and J (**2**).

## RESULTS

The formula for both compounds were elucidated from the mass spectral data, elemental analysis and $^1H$ and $^{13}C$ NMR data of **1** and **2** and their derivatives (See Tables 1 and 2, as well as Experimental Section).

Compound **1**, $C_{22}H_{30}O_8$, gave UV, IR and $^1H$ and $^{13}C$ NMR spectra consistent with the presence of a five-membered ketone conjugated with an exocyclic methylene [240.2 (3.6) nm; 1705 (C=O), 1641 (C=C) $cm^{-1}$, $\delta_H$ 5.93 and 5.47 (each 1H, *s*), $\delta_C$ 116.3 (=$CH_2$), 153.8 (>C=, exo-methylene) and 210.9 (ketone)], which could be represented by partial structure A (Fig. 1). An ester was identified as an acetate by the presence of a three-proton singlets at $\delta_H$ 2.04 (*s*) in addition to the signals at $\delta_C$ 170.8 (*s*) and 20.5 (*q*). A hemiacetal carbon was evident from carbon signal at $\delta_C$ 96.5 (*s*).

Partial structures B and C (Fig. 1) were apparent from $^1H$–$^1H$ COSY and $^1H$–$^{13}C$ COSY spectra. An AX system at $\delta_H$ 3.89 and 2.02 (each 1H, 5.0 Hz, *d*) and $\delta_C$ 73.6 (*d*) and 52.3 (*d*) could be assigned as partial structure D, directly. The remaining signals were assigned to two oxygenated methylenes corresponding to partial structure E (Fig. 1). Combination of partial structures A–E by superposition of the identically numbered quaternary carbon and comparison of the spectral data with those of related *ent*-kaurene structures [3, 4] as well as a consideration of the structures of diterpenoids which have been reported so far from the genus of *Rabdosia* [3], confirmed that compound **1** has the basic skeleton: *ent*-7α-hydroxy-7β, 20-epoxykaur-16-en-15-one (**3**).

Of the $^1H$ NMR data for the two oxygenated methylenes, the signals at $\delta_H$ 4.05 (1H, *dd*, $J = 10.5$, 1.0 Hz) and 3.71 (1H, *dd*, $J = 10.5$, 2.0 Hz) were assigned to $H_a$-20 and $H_b$-20 since a small coupling was observed between H-5

Table 1. $^1H$ NMR data of compounds **1** and **2** (500 MHz, $CD_3OD$, TMS as int. stand.)

| H | 1 | 2 |
|---|---|---|
| 1 | 3.53 *br d* 3.0 | 1.72 *t* 3.0 |
| 1 | — | 1.29 *br d* 14.0 |
| 2 | 1.93 *ddd* 15.0, 3.0, 3.0 | 1.56 *m* |
| 2 | 1.94 *ddd* 15.0, 3.0, 3.0 | 1.79 *dd* 15.0, 3.0 |
| 3 | 3.87 *d* 3.0 | 5.06 *br s* |
| 3 | — | 2.01 *s* |
| 5 | 2.02 *d* 5.0 | 1.90 *d* 5.0 |
| 6 | 3.89 *d* 5.0 | 3.87 *d* 5.0 |
| 9 | 2.03 *ddd* 12.5, 6.0, 2.0 | 1.40 *dd* 14.0, 5.0 |
| 11 | 1.74 *ddd* 14.0, 8.0, 2.0 | 1.73 overlap |
| 11 | 1.81 *ddd* 14.0, 13.0, 12.5 | 1.81 overlap |
| 12 | 2.35 *ddd* 13.5, 8.0, 8.0 | 2.40 *ddd* 13.5, 8.0, 8.0 |
| 12 | 1.46 *ddd* 13.5, 13.0, 7.0 | 1.50 *m* |
| 13 | 3.11 *dd* 7.0, 5.0 | 3.14 *dd* 8.0, 5.0 |
| 14a | 2.20 *d* 12.0 | 2.23 *d* 12.0 |
| 14b | 2.10 *dd* 12.0, 5.0 | 2.14 overlap |
| 17a | 5.93 *s* | 5.99 *s* |
| 17b | 5.47 *s* | 5.53 *s* |
| Me-18 | 1.25 *s* | 1.19 *s* |
| 19a | 4.32 *d* 11.7 | 4.49 *d* 11.7 |
| 19b | 4.37 *d* 11.7 | 4.43 *d* 11.7 |
| Ac-19 | 2.04 *s* | 2.11 *s* |
| 20a | 4.05 *dd* 10.5, 1.0 | 4.05 *br d* 10.5 |
| 20b | 3.71 *dd* 10.5, 2.0 | 3.97 *br d* 10.5 |

Assignments are based on $^1H$–$^1H$, $^{13}C$–$^1H$ COSY and NOESY measurements.

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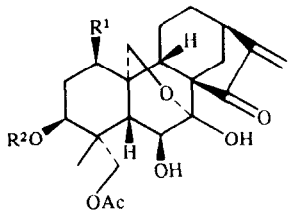
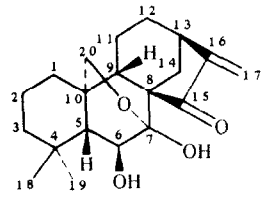


| | $R^1$ | $R^2$ |
|---|---|---|
| **1** | OH | H |
| **2** | H | Ac |
| **4** | OAc | Ac |
| **5** | OH | Ac |



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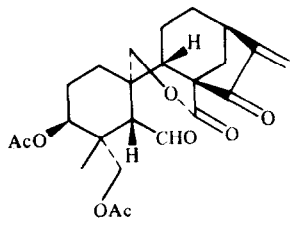

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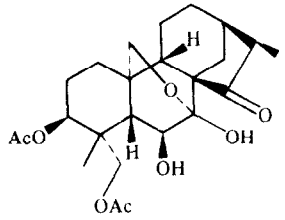


**7**

Table 2. $^{13}C$ NMR data of **1** and **2***

| C | 1† | 2 |
|---|---|---|
| 1 | 66.5 CH–O | 22.1[b] $CH_2$ |
| 2 | 31.2 $CH_2$ | 22.6[b] $CH_2$ |
| 3 | 71.4 CH-O | 72.7[c] CH–O |
| 4 | 41.9[a] C | 41.3 C |
| 5 | 52.3 CH | 57.4 CH |
| 6 | 73.6 CH–O | 73.2[c] CH–O |
| 7 | 96.5 O–C–O | 96.0 O–C–O |
| 8 | 59.8 C | 60.2 C |
| 9 | 45.8 CH | 49.9 CH |
| 10 | 43.0[a] C | 46.3 C |
| 11 | 16.3 $CH_2$ | 16.7 $CH_2$ |
| 12 | 29.7 $CH_2$ | 29.4 $CH_2$ |
| 13 | 35.2 CH | 35.0 CH |
| 14 | 27.0 $CH_2$ | 26.7 $CH_2$ |
| 15 | 210.9 C=O | 210.3 C=O |
| 16 | 153.8 C= | 153.7 C= |
| 17 | 116.3 $H_2C$= | 116.7 $H_2C$= |
| 18 | 22.6 Me | 21.7[d] Me |
| 19 | 67.4 $CH_2$–O | 66.5[c] $CH_2$–O |
| 20 | 66.2 $CH_2$–O | 66.4[c] $CH_2$–O |
| Ac | 170.8 | 170.8 |
| Ac | — | 170.3 |
| Ac | 20.5 | 21.0[d] |
| Ac | — | 20.6[d] |

*Chemical shifts ($\delta$) in ppm relative to pyridine; Multiplicities of signals were determined by INEPT techniques.

†Assignments are based on $^1H$–$^1H$, $^{13}C$–$^1H$ COSY techniques.

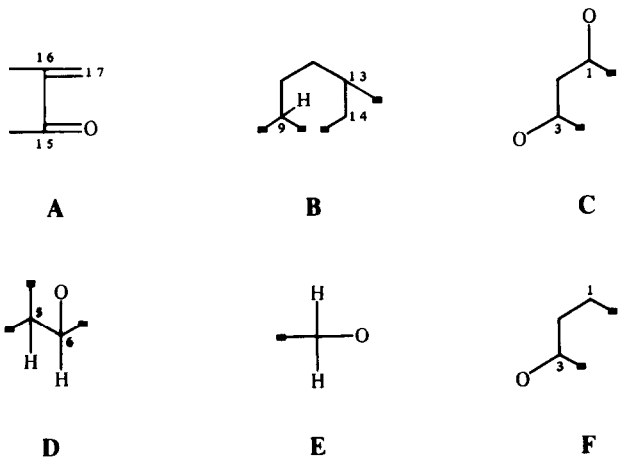
[a–e]Assignments may be interchanged.

and $H_a$-20, and H-9 and $H_b$-20 due to *W*-type long-range coupling [4]. The other signals were attributed to an oxygenated methylene at C-4, because only one methyl signal, $\delta_H$ 1.25 (3H, *s*) and $\delta_C$ 22.6 (*q*), was observed. The positions of the acetoxy group was confirmed by the presence in the 500 MHz $^1H$ NMR spectrum of **1** measured in pyridine-$d_5$ of signals at $\delta_H$ 6.61 (OH, 1H, *d*, 8.0 Hz), 4.40 (H-6, 1H, *dd*, 8.0, 5.3 Hz) and 2.70 ($C_5$-H, 1H, *d*, 5.3 Hz).

Compound **1** was acetylated with acetic anhydride in pyridine to afford diacetate **4** and monoacetate **5**. The hydroxyl groups at C-1 and C-3 in **3** have been acetylated as shown by the downfield shift of $\delta_H$ 3.53 and 3.87 to $\delta_H$ 4.60 and 5.10, respectively. To sum up the above results, the C-19 hydroxy methyl group should be acetylated in **1**.

The unambiguous assignment of all protons of **1** was achieved by a NOESY experiment. Observation of NOEs among $H_b$-20, $H_b$-19 and H-3, as well as between $H_a$-20 and H-1 confirmed that C-19 is axial and that H-1 ($\delta_H$ 3.53) and H-3 ($\delta_H$ 3.87) are equatorial, respectively. Meanwhile, the coupling constant (6.0 Hz) due to H-5 and H-6 and the NOE effect between 18-Me and H-6, H-6 and $H_a$-19 suggested that H-6 has equatorial orientation.

The structure of compound **2**, $C_{24}H_{32}O_8$, was determined on the basis of its INEPT and $^1H$–$^1H$ COSY spectral data to those of **1**. Instead of partial structure C in **1**, partial structure F was revealed, indicating that **2** lacked a hydroxyl group at C-1 or C-3. The signals in the $^1H$ NMR spectrum (500 MHz, pyridine-$d_5$) at $\delta_H$ 7.17 (OH, 1H, *d*, 8.0 Hz), 4.40 (H-6, 1H, *dd*, 8.0, 5.3 Hz) and 2.22 (H-5, 1H, *d*, 5.3 Hz), showed the presence of a free hydroxyl group at C-6. Furthermore, the fact that C-6 and C-7 have free hydroxyl groups was also supported by periodate oxidation of **2** with periodic



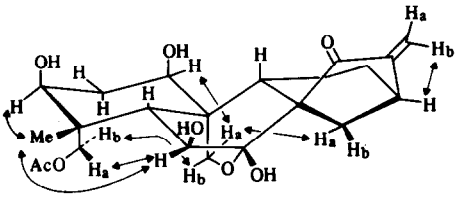

Partial structures A–F

Fig. 1.

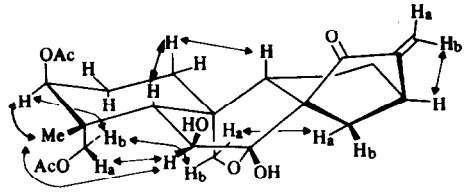
acid in methanol to give a spirolactone-type diterpenoid (**6**) [3]. Consequently, the C-19 hydroxymethyl group and the C-1 or C-3 hydroxyl group had to be acetylated.

The NOEs effect between $H_b$-20, $H_b$-19 and H-3 confirmed that H-3 ($\delta_H$ 5.06 ppm) is equatorial. Moreover, the NOE effects between H-1 (axial, $\delta_H$ 1.72), H-5 and H-9 suggested that they are axial. On the other hand, the NOE effect between $H_a$-14 and $H_a$-20 in both of **1** and **2** established the stereostructure to be as depicted in Fig. 2. In order to establish the absolute configurations of **1** and **2**, a dihydro compound of **2** (**7**) was obtained by catalytic hydrogenation of **2**. The CD spectrum of **7** could be analysed in a manner applicable to that of a bridged-ring (3, 2, 1) structure [5]. The negative Cotton effect indicated that the absolute configuration of the D-ring is the same as that of other similar *ent*-kaurene diterpenes [6]. The stereochemistry of the new methyl at C-16 in **7** was assigned $\beta$-orientation, even if 16*R* stereochemistry, from its chemical shift ($\delta_H$ 1.08, 3H, *d*, 6 Hz; and 2.45, 1H, *quintet*, 6 Hz) [6].

Compounds **1** and **2** inhibited root growth of lettuce seedlings with MICS of less than 200 and 20 ppm, respectively. This effect might be due to the α-methylene-cyclopentanone moiety binding to a sulphydryl enzyme.



**1**



**2**

NOE's observed in maoecrystal I (**1**) and maoecrystal J (**2**)

Fig. 2.

## EXPERIMENTAL

Mps: uncorr. CC: silica gel (Wakogel C-200) and Kieselgel 60 (230–400 mesh, Merck); TLC: Kieselgel 60 $F_{254}$ (Merck); HPLC: ODS $C_{18}$ (6 × 250 mm, detection 240 nm).

*Extraction and isolation of diterpenoids.* Dried and finely powdered leaves of *R. eriocalyx* (Dunn) Hara (3.0 kg), collected on Oct. 1985 at Yanzhonghai, Yunnan, China, were extracted with MeOH (3 × 3 l) at room temp. for 20 days. Filtration and evapn of the solvent yielded 110 g of residue which was dissolved in MeOH–$H_2O$ (1:9) and shaken with 3 × 2 l of $Et_2O$. The $Et_2O$-soln was evapd *in vacuo* to yield 75 g of residue. This was treated (× 2) with activated charcoal in MeOH (1.5 l), filtered and the solvent evapd to yield 44 g of a yellow gum which was subjected to CC over silica gel (700 g). The column was eluted successively with hexane–EtOAc (9:1, 4:1, 3:1, 13:9, and 1:1), EtOAc, and EtOAc–MeOH (4:1). Fractions 27–32 gave 4.2 g crude crystalline **2**. Fractions 36–46 (4.52 g) were bulked and subjected to silica gel CC on Kieselgel 60 (230–400 mesh, Merck) to give 0.1 g crude crystalline **1** from Fr. 17 eluted by $CHCl_3$–MeOH (24:1). The crude crystals were purified by crystallization (hexane–$Me_2CO$ 1:1) to give 0.04 g **1** and 2.8 g **2**.

*Maoecrystal I* (**1**). Mp. 205–206°, $C_{22}H_{30}O_8$ (Found: C, 63.20; H, 7.21. $C_{22}H_{30}O_8$ requires: C, 62.56; H, 7.11%), IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3200, 2950, 1739, 1705, 1641, 1460, 1240; UV $\lambda_{max}^{MeOH}$ nm (log ε) 240.2 (3.6); $^1H$ and $^{13}C$ NMR spectra: see Tables 1 and 2; EIMS 70 eV, *m/z* (rel. int.): 422 $[M]^+$ (100), 404 $[M-H_2O]^+$ (40), 362 $[M-AcOH]^+$ (80), 344 $[M-AcOH-H_2O]^+$ (55) and 326 [M–

$AcOH-2\times H_2O]^+$ (30); CD curve (MeOH) $[\theta]_{243}-10761$, $[\theta]_{338}-2532$.

*Maoecrystal J* (**2**). Mp. 249–250°, $[\alpha]_D=-49.2$ (MeOH; *c* 1.0); $C_{24}H_{32}O_8$ (Found: C, 64.15; H, 7.21. $C_{24}H_{32}O_8$ requires: C, 64.28; H, 7.14%); IR $\nu_{max}^{Nujol}$ $cm^{-1}$: 3760, 3360, 2940, 1741, 1708, 1640, 1460, 1239; $UV_{max}^{MeOH}$ nm (log $\varepsilon$) 239.6 (3.9); $^1H$ and $^{13}C$ NMR spectra: see Tables 1 and 2; EIMS 70 eV *m/z* (rel. int.): 448 $[M]^+$ (40), 430 $[M-H_2O]^+$ (27), 388 $[M-AcOH]^+$ (100), 328 $[M-2\times AcOH]^+$ (55) and 310 $[M-2\times AcOH-H_2O]^+$ (30); CD curve (MeOH) $[\theta]_{240}-18816$, $[\theta]_{338}-4928$.

*Diacetate of* **2** (**4**) *and monoacetate of* **2** (**5**). 1.5 mg of **2** was acetylated with $Ac_2O$ in pyridine at room temp. overnight, then the reaction mixture was poured into ice-$H_2O$ and absorbed to SEP-PAK $C_{18}$. The column was eluted with MeOH, and the crude product was purified by HPLC using $ODS_{18}$ and 50% MeOH to give **5** (800 $\mu$g) and **6** (500 $\mu$g).

**4**, FDMS *m/z*: 507 $[M+H]^+$; $\delta_H$ ($CD_3OD$, 400 MHz): 4.60 (1-H, *t*, 3.0), 2.20 and 2.07 (2-$H_2$, each 1H, *ddd*, 16.0, 3.0, 3.0), 5.11 (3-H, *t*, 3.0), 2.26 and 3.94 (5-H and 6-H, each 1H, *d*, 5.0), 2.06 (9-H, *ddd*, 12.0, 7.0, 1.5), 1.89 and 1.82 (11-$H_2$, each 1H, *m*), 2.40 (12-H, *ddd*, 13.5, 9.0, 9.0), 1.50 (12-H, *ddd*, 13.5, 13.0, 7.0), 3.18 (13-H, overlap), 2.26 (14-H, *d*, 12.0), 2.19 (14-H, *dd*, 12.0, 5.0), 6.00 and 5.55 (17-$H_2$, each 1-H, *br s*), 1.21 (18-$CH_3$, *s*), 4.50 and 4.43 (19-$H_2$, each 1H, *d*, 11.5), 4.18 (20-H, *dd*, 11.0, 1.5), 3.81 (20-H, *dd*, 11.0, 2.5) as well as 3 × AcO: 2.09, 2.11, 2.12 (each 3H, *s*).

**5**, FDMS: *m/z* 465 $[M+H]^+$; $\delta_H$ ($CD_3OD$, 400 MHz): 3.48 (1-H, *t*, 3.0), 2.08 and 2.02 (2-$H_2$, each 1H, *ddd*, 16.0, 3.0, 3.0), 5.06 (3-H, *t*, 3.0), 2.13 and 3.87 (5-H and 6-H, each 1H, *d*, 5.0), 2.00 (9-H, *br d*, 12.0), 1.92 and 1.86 (11-$H_2$, each 1H, *m*), 2.38 (12-H, *ddd*, 13.5, 9.0, 9.0), 1.44 (12-H, *ddd*, 13.0, 13.0, 7.0), 2.21 (14-H, *d*, 12.0), 2.17 (14-H, *dd*, 12.0, 5.0), 5.94 and 5.48 (17-$H_2$, *br s*), 1.17 (18-Me, *s*), 4.45 and 4.39 (19-$H_2$, each 1H, *d*, 11.5), 4.09 (20-H, *dd*, 11.0, 1.5), 3.70 (20-H, *dd*, 11.0, 2.5) as well as 2 × AcO: 2.07 and 2.09 (each 3H, *s*).

*Periodate oxidation of* **2**. **2** (5 mg) was dissolved in MeOH (1 ml) containing $HIO_4$ acid (35 mg) and stirred for 20 hr at room temp. The reaction soln into which 3 ml $H_2O$ was added was evapd. The reaction product was adsorbed to SEP-PAK $C_{18}$ and eluted with MeOH. The solvent was evapd to give a residue (8 mg) which was purified by HPLC using $ODS_{18}$ and 50% MeOH to afford **6** (2 mg).

**6**, FDMS *m/z*: 447 $[M+H]^+$; $\delta_H$ ($CD_3OD$, 400 MHz): 1.52 and 1.80 (H-1 and H-2, overlap), 1.86 and 2.26 (H-1 and H-2, each 1H, *br d*, 15.0), 4.93 (H-3, *s*), 2.99 and 9.90 (H-5 and H-6 [CHO], each 1H, *d*, 5.0), 1.85 and 1.82 (H-9 and H-11, overlap), 1.66 (H-11, *m*), 2.06 and 1.50 (H-12, each 1H, *m*), 3.17 (H-13, *dd*, 9.0, 5.5), 2.34 (H-14, *d*, 13.0), 2.42 (H-14, *dd*, 13.0, 5.5), 6.02 and 5.60 (H-17, each 1H, *s*), 4.18 and 4.22 (H-19, each 1H, *s*), 4.29 and 4.33 (H-20, each 1H, *d*, 10.5) as well as 2 × AcO: 2.08 and 2.15 (each 3H, *s*).

*Hydrogenation of* **2**. **2** (12 mg) was hydrogenated over $Pt_2O$ (5 mg) in MeOH (3 ml) at room temp. for 1.5 hr. The reaction mixture was treated as usual to give crude product, which was purified by HPLC using $ODS_{18}$ and 50% MeOH to give **7** (8 mg).

**7**, FABMS: *m/z* 451 $[M+H]^+$; $\delta_H$ ($CD_3OD$, 400 MHz): 1.21 (1-H, *br d*, 14.0), 1.64 and 1.66 (1-H and 2-H, each 1H, overlap), 1.74 (2-H, *br d*, 15.0), 5.02 (3-H, *t*, 2.5), 1.83 (5-H, *d*, 5.0), 3.75 (6-H, *d*, 5.0), 1.45 (9-H, *dd*, 14.0, 5.0), 1.65 and 1.86 (11-$H_2$, each 1H, overlap), 1.35 and 1.43 (12-$H_2$, each 1H, overlap), 2.55 (13-H, *m*), 2.20 (14-$H_a$, *d*, 12.0), 2.10 (14-$H_b$, *dd*, 12.0, 5.0), 2.45 (16-H, *quintet*, 6.0), 1.08 (17-Me, *d*, 6.0), 1.13 (18-Me, *s*), 4.43 and 4.36 (19-$H_2$, each 1H, 11.5), 3.97 and 3.86 (20-$H_2$, each 1H, 10.5) as well as 2.08 (6H, *s*, 2 × AcO).

*Bioassays*. The root growth inhibitory activities of **1** and **2** were carried out using lettuce seedlings in Hoagland aq. soln. The test doses used were 1, 2, 5, 10, 50, 100, 200, 500 and 1000 ppm. 10 seeds were used in each test.

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