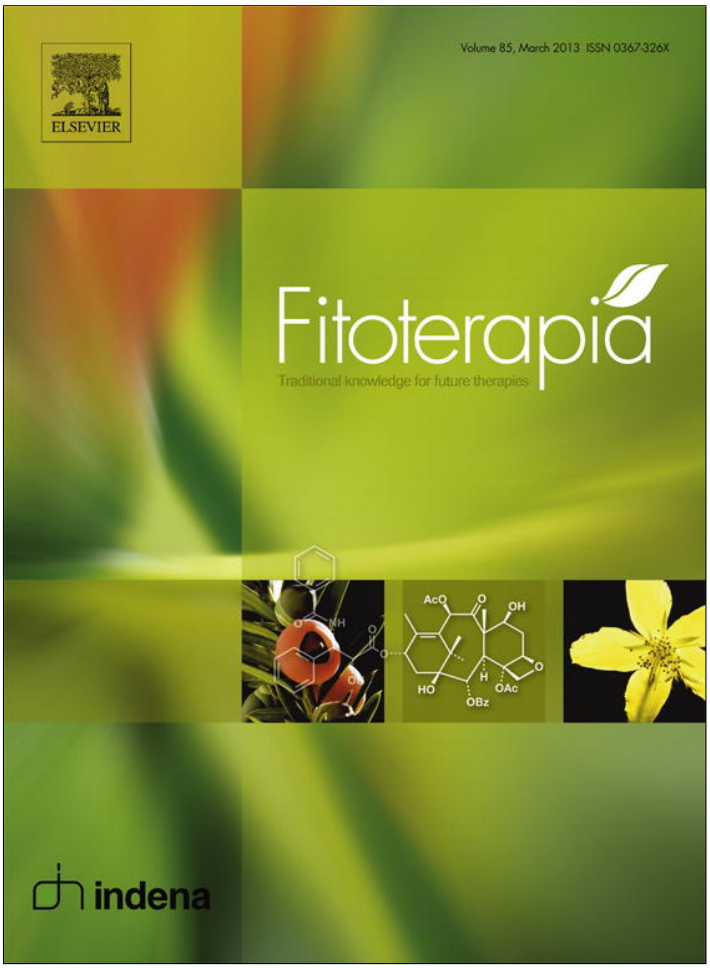

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# Labdane diterpenoids and lignans from *Calocedrus macrolepis*

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ABSTRACT

Three new labdane diterpenoids, calomacrins A–C (**1**–**3**), and a new diaryl butyrolactone-type lignan, calomacrol A (**8**), as well as four known labdane diterpenoids and six known lignans, were isolated from the twigs and leaves of *Calocedrus macrolepis*. Structures of the new compounds were elucidated on the basis of their spectroscopic methods, including 1D and 2D NMR techniques. Compounds **3**–**14** were evaluated for cytotoxicity against HL-60, SMMC-7721, A-549, MCF-7, and SW480 cell lines.



## 1. Introduction

The genus *Calocedrus* (Cupressaceae), containing two species (*Calocedrus decurrens* and *C. macrolepis*) and one variety (*C. macrolepis* var. *formosana* = *C. formosana*), mainly distributed in western north American, Taiwan, and southwestern China [1]. A number of monoterpenoids [2], sesquiterpenoids [2], diterpenoids [2–10], $C_{35}$ terpenoids [11,12], and lignans [13–15] have been isolated from *C. decurrens* and *C. macrolepis* var. *formosana* in the past decades. Many of these compounds exhibited significant cytotoxic [4,8] and anti-inflammatory [6] activities. *C. macrolepis*, an evergreen shrub, mainly grows in Yunnan, Gouzhou, and Guangxi provinces of China [1]. Its wood was used as building material. Up to now, only one article reported chemical study of *C. macrolepis* which described the components of essential oil from its leaves [16]. Our current phytochemical investigation on the twigs and leaves of *C. macrolepis* led to the isolation of three new labdane diterpenoids, calomacrins A-C (**1**–**3**), and a new diaryl butyrolactone-type lignan, calomacrol A (**8**), together with four known labdane diterpenoids (**4**–**7**) and six known lignans (**9**–**14**). Reported herein are the isolation and structure elucidation of the new compounds, and the cytotoxic activity evaluation of the selected isolates.

## 2. Experimental

### 2.1. Gernal

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. IR spectra were obtained by a Bruker Tensor 27 spectrophotometer with KBr pellets. 1D and 2D spectra were run on a Bruker AM-400 or Avance III 600 spectrometer with TMS as the internal standard. Chemical shifts ($\delta$) were expressed in ppm with reference to the solvent signals. ESIMS and HRESIMS were performed on an API QSTAR time-of-flight spectrometer. EIMS and HREIMS were recorded on an Waters AutoSpec Premier P776 spectrometer. Silica gel (100–200 and 200–300 mesh, Qingdao Marine Chemical Co. Ltd., Qingdao, China) and Sephadex LH-20 (Amersham Pharmacia Biotech, Sweden) were used for

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Column chromatography (CC). MPLC was performed on a Lisui EZ Purify III System including pump manager P03, detector modules P02, and fraction collector P01 (Shanghai Li Sui Chemical Engineering Co., Ltd., China) and columns packed with MCI gel (75–150 μm; Mitsubishi Chemical Corporation, Japan). Semi-preparative HPLC was performed on an Agilent 1260 apparatus equipped with a UV detector and a Zorbax SB-C-18 (Agilent, 9.4 mm × 25 cm) column. Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% $H_2SO_4$ in EtOH.

### 2.2. Plant material

The twigs and leaves of *C. macrolepis* was collected from Kunming Botany Garden, Yunnan province, People's Republic of China, in December 2010, and identified by Prof. X. Gong (one of the authors), Kunming Institute of Botany. A voucher specimen (KIB20101224c) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

### 2.3. Extraction and isolation

The air-dried and powdered twigs and leaves of *C. macrolepis* (15 kg) were extracted three times with 95% EtOH at room temperature. The filtrate was concentrated under reduced pressure to yield a residue, which was partitioned between $H_2O$ and EtOAc. The EtOAc fraction (620 g) was chromatographed on a silica gel column with a gradient elution of petroleum ether–acetone (1:0 to 0:1) to give five fractions (1–5). Fraction 2 (110 g) was applied to silica gel CC eluted with petroleum ether–EtOAc (from 20:1 to 7:3) to afford three subfractions (2a–2c). Subfraction 2b was further purified by recrystallization to obtain **5** (4.5 g). Fraction 3 (85 g) was separated by MPLC eluting with MeOH–$H_2O$ (from 75:25 to 100:0) to provide five subfractions, 3a–3e. Subfraction 3b was subjected to silica gel CC ($CHCl_3$–actone, 9:1), followed by

**Table 1**
$^1H$ and $^{13}C$ NMR spectroscopic data of **1**–**3** ($\delta$ in ppm).

| No. | 1[a] | | 2[a] | | 3[b] | |
|---|---|---|---|---|---|---|
| | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ |
| 1α | 0.98 (m) | 40.3 t | 0.98 (m) | 40.3 t | 1.11 (m) | 40.3 t |
| 1β | 1.68 (m) | | 1.68 (m) | | 1.84 (m) | |
| 2α | 1.36 (m) | 18.9 t | 1.34 (m) | 18.9 t | 1.49 (m) | 21.1 t |
| 2β | 1.65 (m) | | 1.63 (m) | | 1.89 (m) | |
| 3α | 0.97 (m) | 36.9 t | 0.94 (m) | 36.8 t | 1.06 (m) | 39.2 t |
| 3β | 1.83 (m) | | 1.76 (m) | | 2.12 (m) | |
| 4 | | 38.0 s | | 37.8 s | | 45.1 s |
| 5 | 1.14 (br d 12.0) | 57.4 d | 1.14 (br d 11.4) | 57.4 d | 1.35 (m) | 57.3 d |
| 6α | 1.76 (m) | 21.5 t | 1.74 (m) | 21.5 t | 2.00 (m) | 27.4 t |
| 6β | 1.38 (m) | | 1.38 (m) | | 1.88 (m) | |
| 7α | 1.74 (m) | 45.7 t | 1.73 (m) | 45.7 t | 1.93 (m) | 39.7 t |
| 7β | 2.03 (m) | | 2.04 (m) | | 2.41 (m) | |
| 8 | | 73.4 s | | 73.4 s | | 149.1 s |
| 9 | 1.44 (m) | 62.6 d | 1.44 (m) | 62.6 t | 1.67 (m) | 56.9 d |
| 10 | | 39.5 s | | 39.5 s | | 41.4 s |
| 11a | 1.57 (m) | 27.6 t | 1.58 (m) | 27.6 t | 1.61 (m) | 22.9 t |
| 11b | 2.08 (m) | | 2.10 (m) | | 1.77 (m) | |
| 12a | 2.66 (m) | 29.4 t | 2.67 (m) | 29.4 t | 2.10 (m) | 25.3 t |
| 12b | 2.98 (m) | | 2.99 (m) | | 2.40 (m) | |
| 13 | | 127.3 s | | 127.3 s | | 139.1 s |
| 14 | 6.50 (br s) | 112.3 d | 6.52 (br s) | 112.3 d | 6.95 (br s) | 144.6 d |
| 15 | 7.53 (br s) | 139.8 d | 7.54 (br s) | 139.8 d | 5.91 (d 4.5) | 103.5 d |
| 16 | 7.59 (s) | 143.6 d | 7.60 (s) | 143.6 d | | 173.6 s |
| 17a | 1.30 (s) | 25.0 q | 1.32 (s) | 25.0 q | 4.62 (br s) | 107.1 t |
| 17b | | | | | 4.89 (br s) | |
| 18 | 1.05 (s) | 28.1 q | 1.02 (s) | 28.1 q | 1.19 (s) | 29.5 q |
| 19a | 4.18 (d 11.0) | 67.2 t | 4.13 (d 11.0) | 67.1 t | | 181.2 s |
| 19b | 4.55 (d 11.0) | | 4.53 (d 11.0) | | | |
| 20 | 0.84 (s) | 16.7 q | 0.86 (s) | 16.7 q | 0.64 (s) | 13.3 q |
| 1′ | | 126.6 s | | 127.1 s | | |
| 2′ | 7.67 (d 8.6) | 131.3 d | 8.11 (d 8.7) | 134.2 d | | |
| 3′ | 7.20 (d 8.6) | 117.4 d | 7.22 (d 8.7) | 116.5 d | | |
| 4′ | | 162.0 s | | 161.1 s | | |
| 5′ | 7.20 (d 8.6) | 117.4 d | 7.22 (d 8.7) | 116.5 d | | |
| 6′ | 7.67 (d 8.6) | 131.3 d | 8.11 (d 8.7) | 134.2 d | | |
| 7′ | 8.04 (d 15.9) | 145.7 d | 7.02 (d 12.9) | 144.5 d | | |
| 8′ | 6.74 (d 15.9) | 115.8 d | 6.06 (d 12.9) | 116.8 d | | |
| 9′ | | 168.3 s | | 167.7 s | | |
| –OEt | | | | | 3.75 (m) | 66.7 t |
| | | | | | 3.86 (m) | |
| | | | | | 1.24 (t 7.1) | 15.4 q |

[a] Recorded in $C_5D_5N$ at 600 MHz for $^1H$ and 150 MHz for $^{13}C$.
[b] Recorded in $CD_3OD$ at 500 MHz for $^1H$ and 125 MHz for $^{13}C$.

Sephadex LH-20 (MeOH) to yield **3** (8 mg) and **4** (15 mg). Subfraction 3c was separated by silica gel CC eluted with ($CHCl_3$–acetone) (from 9:1 to 7:3) to afford **6** (2.3 g) and a mixture, which was further purified by semipreparative HPLC (MeCN–$H_2O$, 57:43) to afford **1** (1.1 mg) and **2** (1.2 mg). Compound **7** (7 mg) was obtained from subfraction 3d by repeated silica gel CC, eluted with $CHCl_3$–acetone (from 9:1 to 6:4) and petroleum ether–acetone (8:2 to 6:4). Fraction 4 (65 g) was chromatographed by MPLC eluting with MeOH–$H_2O$ (from 30:70 to 100:0) to provide six subfractions, 4a–4f. Subfraction 4b was separated by silica gel CC (petroleum ether–acetone, 8:2 to 6:4) to yield **12** (180 mg). Subfraction 4c was subjected to silica gel CC eluted with $CHCl_3$–acetone (from 8:2 to 6:4), followed by Sephadex LH-20 (MeOH), and further purified by semipreparative HPLC (MeOH–$H_2O$, 53:47) to afford **9** (15 mg), **10** (6 mg), and **11** (80 mg). Subfraction 4d was subjected to Sephadex LH-20 (MeOH) and then semipreparative HPLC (MeOH–$H_2O$, 40:60) to afford **8** (29 mg). Subfraction 4e was separated further on silica gel CC (petroleum ether–acetone, 8:2 to 6:4) and Sephadex LH-20 (MeOH) to yield **13** (6 mg) and **14** (20 mg).

### 2.4. Spectroscopic data

**Calomacrin A** (**1**): white powder; [α]20.8 D +5.60 (*c* 0.16, MeOH); UV (MeOH) $\lambda_{max}$ (log ε): 312 (4.46), 210 (4.32) nm; IR (KBr) $\nu_{max}$ 3425, 2926, 2853, 1681, 1631, 1605, 1514, 1451, 1385, 1370, 1263, 1167, 1022, 873, 723 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, see Table 1; EIMS: *m/z* 466 $[M]^+$; HREIMS $[M]^+$ *m/z* 466.2708 (calcd for $C_{29}H_{38}O_5$, 466.2719).

**Calomacrin B** (**2**): white powder; [α]22.8 D +3.89 (*c* 0.34, MeOH); UV (MeOH) $\lambda_{max}$ (log ε): 309 (4.45), 208 (4.56) nm; IR (KBr) $\nu_{max}$ 3424, 2926, 2853, 1689, 1629, 1605, 1513, 1451, 1385, 1277, 1166, 873, 722, 627 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, see Table 1; EIMS: *m/z* 466 $[M]^+$; HREIMS $[M]^+$ *m/z* 466.2709 (calcd for $C_{29}H_{38}O_5$, 466.2719).

**Calomacrin C** (**3**): colorless oil; [α]18.5 D +68.11 (*c* 0.23, MeOH); UV (MeOH) $\lambda_{max}$ (log ε): 202 (4.34) nm; IR (KBr) $\nu_{max}$ 3427, 2935, 1769, 1692, 1643, 1468, 1449, 1374, 1343, 1181, 1022, 938 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, see Table 1; positive ESIMS: *m/z* 399 $[M+Na]^+$; negative HRESIMS $[M-H]^-$ *m/z* 375.2172 (calcd for $C_{22}H_{31}O_5$, 375.2171).

**Calomacrol A** (**8**): colorless oil; [α]21.1 D –202.42 (*c* 0.09, MeOH); UV (MeOH) $\lambda_{max}$ (log ε): 284 (4.00), 231 (4.21), 203 (4.89) nm; IR (KBr) $\nu_{max}$ 3441, 2922, 1768, 1630, 1516, 1503, 1491, 1444, 1366, 1273, 1244, 1204, 1154, 1126, 1036 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, see Table 2; positive ESIMS: *m/z* 411 $[M+Na]^+$; negative HRESIMS $[M-H]^-$ *m/z* 387.1078 (calcd for $C_{20}H_{19}O_8$, 387.1079).

### 2.5. Cytotoxicity assay

The cytotoxicity of compounds **3**–**14** against human myeloid leukemia (HL-60), hepatocellular carcinoma (SMMC-7721), lung cancer (A-549), breast cancer (MCF-7), and colon cancer (SW-480) cell lines was assessed using the MTT method [17]. Cells were plated in 96-well plates 12 h before treatment and continuously exposed to different concentrations of compounds. After 48 h, 20 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution were added to each well, which were incubated for another 4 h. Then 20% SDS (100 μL) was added to each well. After 12 h at room temperature, the OD value of each well was recorded at 595 nm. The $IC_{50}$ value of each compound was calculated by the Reed and Muench method [18].

**Table 2**
$^1H$ and $^{13}C$ NMR spectroscopic data of compound **8** (δ in ppm).

| Pos. | **8**[a] | | **8**[b] | |
|---|---|---|---|---|
| | $\delta_H$ (*J* in Hz) | $\delta_C$ | $\delta_H$ (*J* in Hz) | $\delta_C$ |
| 1 | | 126.4 s | | 128.2 s |
| 2 | 7.01 (d 1.7) | 115.6 d | 7.03 (d 1.8) | 116.3 d |
| 3 | | 146.7 s | | 148.3 s |
| 4 | | 144.9 s | | 146.3 s |
| 5 | 6.67 (d 8.1) | 114.6 d | 6.71 (d 8.1) | 115.5 d |
| 6 | 6.81 (dd 8.1, 1.7) | 123.8 d | 6.85 (dd 8.1, 1.8) | 125.2 d |
| 7a | 2.90 (d 14.2) | 35.6 t | 3.02 (d 14.4) | 37.7 t |
| 7b | 2.95 (d 14.2) | | 3.06 (d 14.4) | |
| 8 | | 78.5 s | | 80.4 s |
| 9 | | 177.8 s | | 180.2 s |
| 1′ | | 130.0 s | | 131.2 s |
| 2′ | 6.68 (d 1.6) | 110.4 d | 6.67 (d 1.6) | 111.6 d |
| 3′ | | 146.5 s | | 148.9 s |
| 4′ | | 145.6 s | | 147.8 s |
| 5′ | 6.78 (d 8.0) | 107.8 d | 6.70 (d 7.9) | 108.8 d |
| 6′ | 6.54 (dd 8.0, 1.6) | 123.0 d | 6.58 (dd 7.9, 1.6) | 124.3 d |
| 7′a | 2.13 (d 14.1) | 36.8 t | 2.35 (d 14.3) | 38.6 t |
| 7′b | 2.51 (d 14.1) | | 2.65 (d 14.3) | |
| 8′ | | 76.9 s | | 78.9 s |
| 9′a | 3.60 (d 9.2) | 74.2 t | 3.64 (d 9.4) | 75.8 t |
| 9′a | 4.21 (d 9.2) | | 4.26 (d 9.4) | |
| $-OCH_2O-$ | 5.95 (2H br s) | 100.6 t | 5.88 (2H br s) | 102.1 t |
| $OCH_3$ | 3.74 (s) | 55.5 q | 3.86 (s) | 56.4 q |
| 4-OH | 8.78 (s) | | | |
| 8-OH | 6.24 (s) | | | |
| 8′-OH | 5.34 (s) | | | |

[a] Recorded in DMSO-$d_6$ at 600 MHz for $^1H$ and 150 MHz for $^{13}C$.
[b] Recorded in $CD_3OD$ at 400 MHz for $^1H$ and 100 MHz for $^{13}C$.

## 3. Results and discussion

### 3.1. Chemistry

Calomacrin A (**1**) was obtained as a white powder. Its molecular formula, $C_{29}H_{38}O_5$, was established by HREIMS at *m/z* 466.2708 $[M]^+$ (calcd *m/z* 466.2719), corresponding to 11° of unsaturation. The IR spectrum showed absorptions of hydroxy (3425 $cm^{-1}$) and double bond (1631 $cm^{-1}$) groups. The 1D NMR spectra (Table 1) showed 29 carbon signals for a diterpenoid unit together with nine characteristic signals for a (*E*)-coumaroyl moiety [$\delta_H$ 7.67 (2H, d, *J* = 8.6 Hz, H-2′ and H-6′), 7.20 (2H, d, *J* = 8.6 Hz, H-3′ and H-5′), 8.04 (1H, d, *J* = 15.9 Hz, H-7′), and 6.74 (1H, d, *J* = 15.9 Hz, H-8′); $\delta_C$ 126.6 (C-1′), 131.3 (C-2′ and C-6′), 117.4 (C-3′ and C-5′), 162.0 (C-4′), 145.7 (C-7′), 115.8 (C-8′), 168.3 (C-9′)]. The remaining diterpenoid unit consisted of three methyls, seven methylenes, two methines, two quaternary carbons, one oxygenated methylene [$\delta_H$ 4.18 (1H, d, *J* = 11.0 Hz, H-19a), 4.55 (1H, d, *J* = 11.0 Hz, H-19b); $\delta_C$ 67.2 (C-19)], one oxygenated quaternary carbon [$\delta_C$ 73.4 (C-8)], and a β-substituted funan ring [$\delta_H$ 6.50

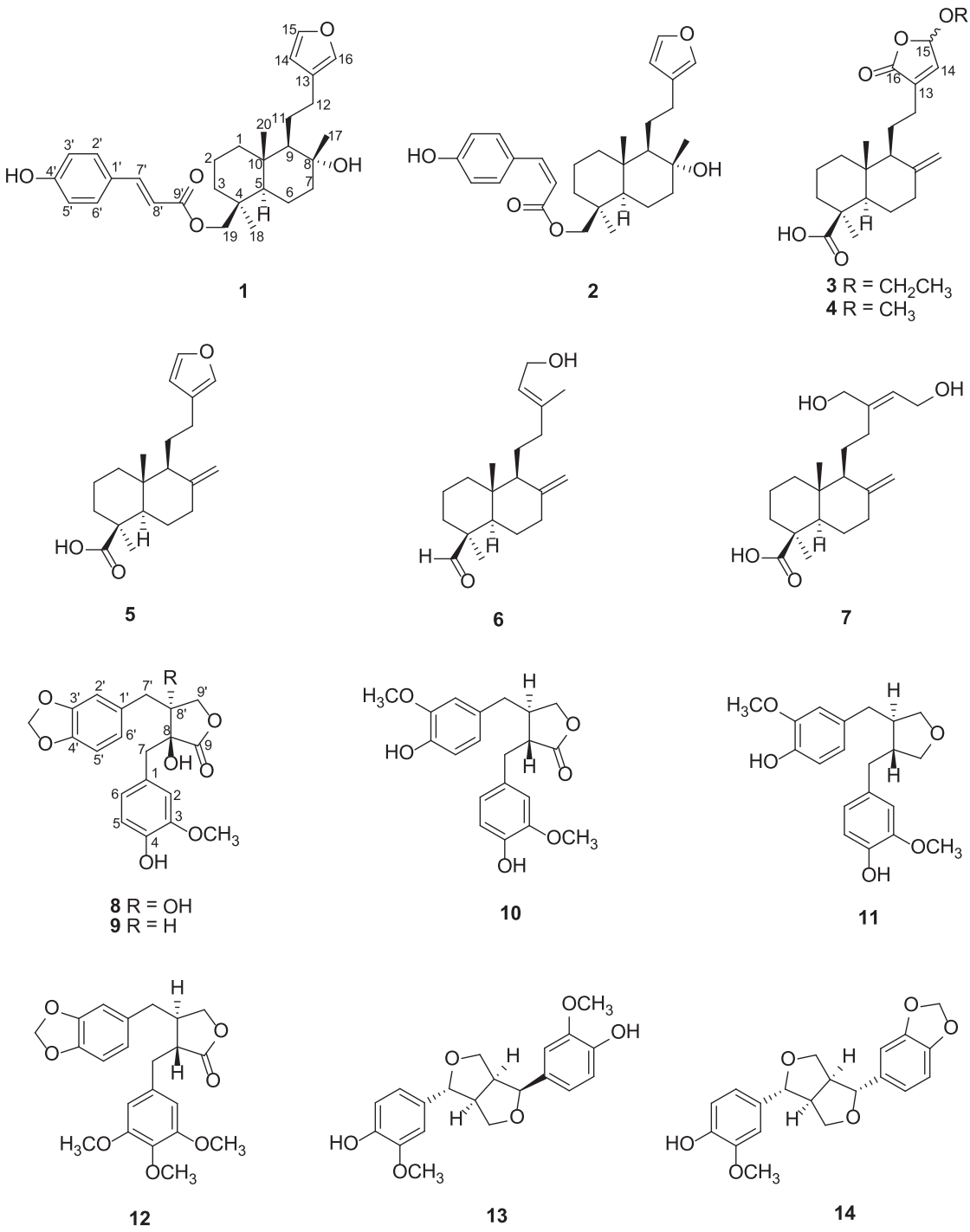


**Fig. 1.** Structures of compounds **1**–**14**.

(1H, br s, H-14), 7.53 (1H, br s, H-15), 7.59 (1H, s, H-16); $\delta_C$ 127.3 (C-13), 112.3 (C-14), 139.8 (C-15), 143.6 (C-16)]. The above NMR spectral features were similar to those of 15,16-epoxylabda-13(16),14-dien-8α,19-diol [19], a labdane diterpenoid, except for the nine more signals for the (*E*)-coumaroyl group. The obvious substituted effects of carbon signal due to downfield shift at C-19 suggested that the additional (*E*)-coumaroyl group was linked to the C-19 position of the diterpenoid unit of **1**. This deduction was confirmed by HMBC correlations (Fig. 2) of H-19a and H-19b with the ester carbonyl carbon at $\delta_C$ 168.6 of the (*E*)-coumaroyl group.

The relative configuration of **1** was established by a ROESY experiment (Fig. 3). The ROESY correlations of H-5/H-9 and H-5/$H_3$-18 indicated the α-orientation of H-5, H-9, and $H_3$-18. Meanwhile, the correlations of $H_3$-17/$H_3$-20 and $H_2$-19/$H_3$-20 suggested the β-orientation of these groups. Therefore, the structure of **1** was assigned as 8α-hydroxy-15,16-epoxylabda-13(16),14-dien-19-yl (*E*)-coumarate.

Calomacrin B (**2**), a white powder, had molecular formula $C_{29}H_{38}O_5$ according to its HREIMS at *m/z* 466.2709 $[M]^+$ (calcd *m/z* 466.2719). Detailed comparison of the NMR data (Table 1) of **2** and **1** indicated that these two compounds were

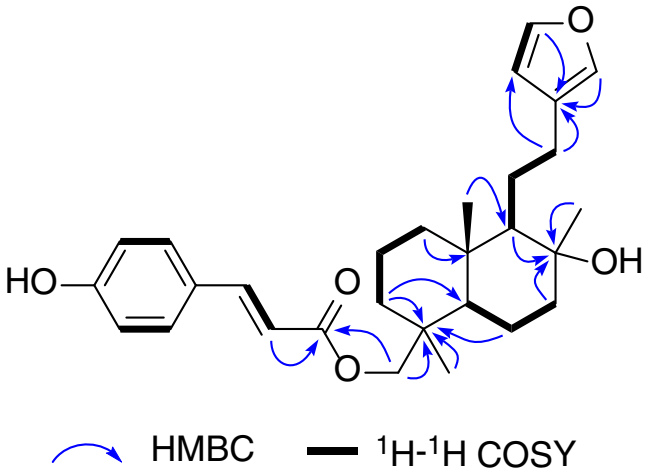


**Fig. 2.** Key HMBC and $^1$H–$^1$H COSY correlations of **1**.

similar. The only difference was that the (*E*)-coumaroyl moiety in **1** was replaced by an (*Z*)-coumaroyl moiety [$\delta_H$ 8.11 (2H, d, $J$ = 8.7 Hz, H-2′ and H-6′), 7.22 (2H, d, $J$ = 8.7 Hz, H-3′ and H-5′), 7.02 (1H, d, $J$ = 12.9 Hz, H-7′), and 6.06 (1H, d, $J$ = 12.9 Hz, H-8′] in **2**, which was also located at C-19 as deduced by the HMBC correlations from protons at $\delta_H$ 4.13 (H-19a) and 4.53 (H-19b) to the carbonyl carbon at $\delta_C$ 167.7 of the (*Z*)-coumaroyl group. The relative configuration of the diterpenoid unit of **2** was assigned as being the same as that of **1** according to the 1D NMR data and ROESY experiment. Hence, compound **2** was elucidated as 8α-hydroxy-15,16-epoxylabda-13(16),14-dien-19-yl (*Z*)-coumarate.

Calomacrin C (**3**) was obtained as a colorless oil. Its molecular formula was determined to be $C_{22}H_{32}O_5$ on the basis of negative HRESIMS at *m/z* 375.2172 $[M-H]^-$ (calcd *m/z* 375.2171). The IR absorptions at 3427, 1769, 1692 $cm^{-1}$ indicated the presence of hydroxy and carbonyl groups. The $^1$H NMR spectrum (Table 1) showed signals for an olefinic proton [$\delta_H$ 6.95 (1H, br s, H-14)], two exocyclic olefinic protons [$\delta_H$ 4.62 (1H, br s, H-17a), 4.89 (1H, br s, H-17b)], two tertiary methyl groups [$\delta_H$ 1.19 (3H, s, $H_3$-18), 0.64 (3H, s, $H_3$-20)], and an ethoxy group [$\delta_H$ 3.75 (1H, m), 3.86 (1H, m), 1.24 (3H, t, $J$ = 7.1 Hz)]. The $^{13}$C NMR and DEPT spectrum of **3** (Table 1) displayed signals for 22 carbons, including three methyls, nine methylenes (including one oxygenated and one $sp^2$ methylenes), four methines (including one acetal and one $sp^2$ methines), and six quaternary carbons (including two carbonyl and two $sp^2$ carbons). These data suggested that **3** was a labdane diterpenoid with an ethoxy group. Analysis of the $^1$H and $^{13}$C NMR data (Table 1) of **3** revealed that they were similar to those of 15-methoxypinusolidic acid (**4**) [5], a known compound which was also isolated from this plant in present study. The main difference between **3** and **4** was the presence of an ethoxy group in **3** rather than a methoxy functionality in **4**, as inferred from the HMBC correlations of the proton signals at $\delta_H$ 3.75 and 3.86 with the acetal carbon signal at $\delta_C$ 103.5 (C-15). The ROESY correlations of H-5/$H_3$-18, and H-5/

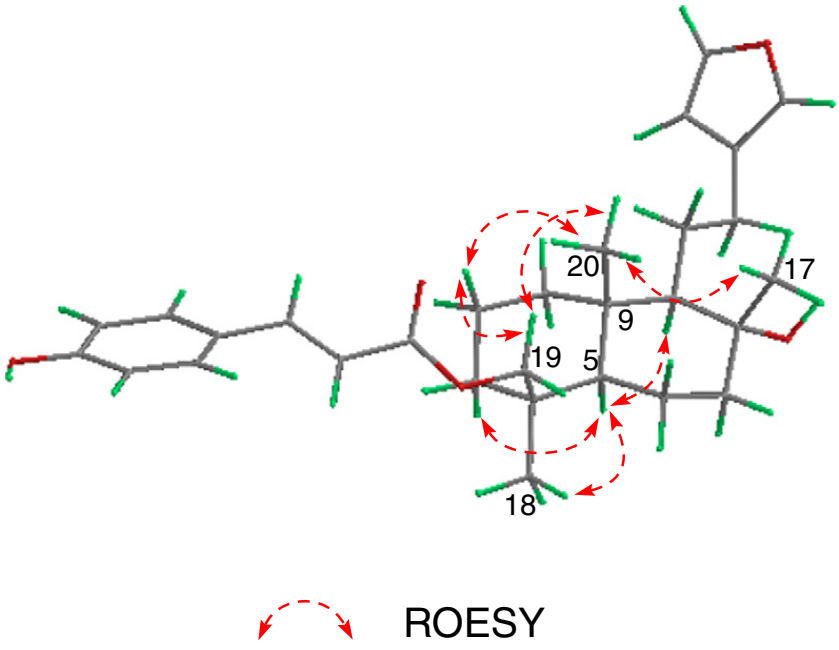


**Fig. 3.** Key ROESY correlations of **1**.

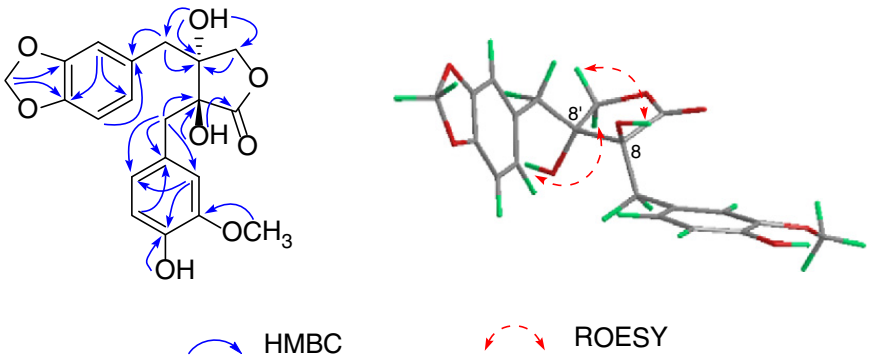


**Fig. 4.** Key HMBC and ROESY correlations of **8**.

H-9 indicated that these protons were cofacial and arbitrarily assigned as $\alpha$-orientation. In addition, the ROESY cross peaks of H-11b/$H_3$-20 revealed that $H_3$-20 were $\beta$-oriented. Compounds **3** and **4** could be artifacts because 95% EtOH and methanol were used during extraction and isolation. Thus, the structure of **3** was identified as 15-ethoxypinusolidic acid.

Calomacrins C (**8**) was isolated as a colorless oil. The negative HRESIMS ($[M-H]^-$ *m/z* 387.1078, calcd 387.1079) suggested its molecular formula of $C_{20}H_{20}O_8$, indicative of 11° of unsaturation. The IR spectrum showed absorptions bands characteristic of hydroxy (3441 $cm^{-1}$), carnonyl (1768 $cm^{-1}$), and aromatic rings (1630, 1516, 1503 $cm^{-1}$). Analysis of its $^1H$ NMR (DMSO-$d_6$) data (Table 2) revealed the presence of two ABX benzene rings [$\delta_H$ 7.01 (1H, d, $J=1.7$ Hz, H-2), 6.67 (1H, d, $J=8.1$ Hz, H-5), 6.81 (1H, dd, $J=8.1$, 1.7 Hz, H-6), 6.68 (1H, d, $J=1.6$ Hz, H-2′), 6.78 (1H, d, $J=8.0$ Hz, H-5′), and 6.54 (1H, dd, $J=8.0$, 1.6 Hz, H-6′)]. The $^{13}C$ NMR and DEPT (DMSO-$d_6$) spectra (Table 2) showed signals due to twelve aromatic carbons, two methylenes, one oxygenated methylene [$\delta_C$ 74.2 (C-9′)], two oxygenated quaternary carbons [$\delta_C$ 78.5 (C-8) and 76.9 (C-8′)], one ester group [$\delta_C$ 177.8 (C-9)], one methylenedioxy group ($\delta_C$ 100.6), and one methoxy group ($\delta_C$ 55.5). The NMR data of **8** were similar to those of 8-hydroxypluviatolide [20], except for the presence of one more hydroxy group in **8**. The HMBC correlations (Fig. 4) from 8′-OH ($\delta_H$ 5.34), $H_2$-7′ ($\delta_H$ 2.13, 2.51), and $H_2$-9′ ($\delta_H$ 3.60, 4.21) to C-8′, and from 8′-OH to C-7′ ($\delta_C$ 36.8) indicated the additional hydroxy group was located at C-8′. In the ROESY spectrum (Fig. 4), the correlations of 8-OH/H-9′$\beta$, and 8′-OH/H-9′$\alpha$ suggested the *trans* configuration of 8-OH and 8′-OH. Consequently, the structure of **8** was determined as 8,8′-dihydroxypluviatolide.

The ten known compounds (Fig. 1) were readily identified as 15-methoxypinusolidic acid (**4**) [5], lambertianic acid (**5**) [21], agatholal (**6**) [3], 15,16-dihydroxy-8(17),13 (*E*)-labdadien-19-oic acid (**7**) [22], 8-hydroxypluviatolide (**9**) [20], matairesinol (**10**) [23], shonanin (**11**) [14], yatein (**12**) [24], epipinoresinol (**13**) [25], piperitol (**14**) [26], respectively, by comparison of their spectroscopic data with those reported in literature. All of these compounds were isolated from *C. macrolepis* for the first time. In addition, compounds **4**–**6**, **10**–**12** have been previously reported from *C. formosana* [4,14,15]. These finds indicated a close relationship between *C. macrolepis* and *C. formosana* from the chmotaxonomic point of view.

**Table 3**
Cytotoxicity of compounds **6** and **12** against selected tumor cell lines ($IC_{50}$ μM).

| Compound | HL-60 | SMMC-7721 | A-549 | MCF-7 | SW-480 |
|---|---|---|---|---|---|
| **6** | 16.32 | >40 | >40 | >40 | >40 |
| **12** | 0.69 | 0.86 | 1.09 | 1.24 | 1.06 |
| Cisplatin[a] | 1.29 | 7.18 | 5.16 | 16.07 | 14.70 |

[a] Positive control.

### 3.2. Biological activity

Due to the limited amount of material available, compounds **1** and **2** were not tested for its cytotoxicity. The other compounds **3**–**14** were tested for their cytotoxicity against HL-60, SMMC-7721, A-549, MCF-7, and SW480 cell lines using the MTT method as previously reported [17], with cisplatin (sigma) as the positive control. Compound **6** exhibited selective cytotoxicity against HL-60 cell line, while compound **12** showed significant cytotoxicity against all the test cell lines. (Table 3). The remaining compounds were noncytotoxic in these test systems ($IC_{50}>40$ μM). Yatein (**12**) was a diarylbutane lignan with potent cytotoxic activity and had a structure similar to those of podophyllotoxin. These results suggested that yatein might be a potential candidate for developing anticancer agents from natural resources.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2013.01.011.

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