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# Constituents and bioactivity of the tubers of *Euphorbia sessiliflora*☆

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**Abstract**

The diterpene *ent*-12-hydroxy-12[*R*]-abieta-8(14),13(15)-dien-16,12-olide was isolated from the tubers of *Euphorbia sessiliflora* Roxb., together with four known *ent*-abietadienolides, four known cycloartane triterpenes and ellagic acid-β-D-glucopyranoside. Two of these metabolates displayed moderate antibacterial activities. © 2000 Elsevier Science Ltd. All rights reserved.

*Keywords:* *Euphorbia sessiliflora*; Euphorbiaceae; *ent*-Abietadienolide; *ent*-12-Hydroxy-12[*R*]-abieta-8(14),13(15)-dien-16,12-olide; Antibacterial activity

## 1. Introduction

*Euphorbia sessiliflora* Roxb., known in Thai as ''Khaoo Khaa'' or ''Wann Phra Chim'', is indigenous to Thailand. The plant grows to about 30 cm in height, with ovoid leaves approximately 5 cm × 3 cm in size (Pongboonrod, 1965). This herb has been used as a folk medicine for treatment of yaws and applied as a poultice to boils. Its latex is extremely caustic and poisonous. The chloroform extract of the tubers of *E. sessiliflora* exhibits inhibitory effects against the growth of several strains of bacteria and yeast. Repeated column chromatography of this extract resulted the isolation of a new *ent*-abietane (**1**) and 13 known compounds. We now report the structures of these compounds.

## 2. Results and discussion

The known compounds were confirmed to be jolkinolide A (**2**) (Lal, Cambie, Rutledge & Woodgate, 1990; Uemura & Hirata, 1972), jolkinolide B (**3**) (Uemura & Hirata, 1972), a mixture of β-sitosterol and stigmasterol, cycloart-25-en-3β, 24-diol (**4**) (Anjaneyulu, Rao & Connolly, 1985), cycloart-23Z-en-3β,25-diol (**5**) (Greca, Fitorentino & Previtera, 1994), 25-methoxy-cycloart-23E-en-3β-ol (**6**) (Djerassi & McCrindle, 1962), *ent*-11α-hydroxyabieta-8(14),13(15)-dien-16,12α-olide (**7**) (Lal et al., 1990), caudicifolin (**8**) (Satti et al., 1986), cycloartan-3β,24,25-triol (**9**) (Teresa, Urones, Marcos, Basabe, Cuadrado & Moro, 1987), a mixture of 3-β-D-glucopyranosides of sitosterol and stigmasterol (Ruangrungsi, Aukkanibutra, Phadungcharoen, Lange & Lee, 1987), and 3,3′,4-tri-*O*-methylellagic acid-4′-*O*-β-D-glucopyranoside (Do Khac, Tran-Van, Campos, Lallemand & Fetizon, 1990); their physical and spectral data were almost identical with those published in the literature. Compound (**6**) is presumed to be an artifact of the isolation procedure; its 3-acetate derivative was isolated from Spanish Moss

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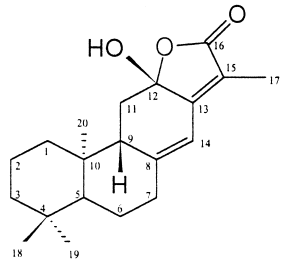
Table 1
Bioassay results[a]

| Test sample | Solvent | Concentration (μg/ml) | Activity |
|---|---|---|---|
| Chloroform extract | Acetone | 500 | Active[b] |
| | | 250 | Not active |
| Jolkinolide A (**2**) | 34% DMSO/MeOH | 50 | Active[c] |
| | | 25 | Not active |
| *ent*-11α-Hydroxyabieta-8(14),13(15)-dien-16,12α-olide (**7**) | 34% DMSO/MeOH | 12.5 | Active[d] |
| Gentamicin | $H_2O$ | 0.5[e] | Active |

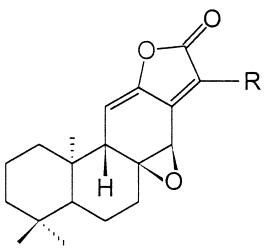
[a] Mixture of sitosterol and stigmasterol (50 μg/ml), *ent*-12-hydroxy-12[*R*]-abieta-8(14),13(5)-dien-16,12-olide (**1**) (50 μg/ml), cycloart-23*Z*-en-3β,25-diol (**5**) (25 μg/ml), caudicifolin (**8**) (25 μg/ml) were also tested but not active.
[b] Active with *Bacillus cereus*, *Micrococcus flavas* and *Neisseria sicca* only.
[c] Active with *Moraxella catarrhalis* only.
[d] Active with *Bacillus cereus*, *Micrococcus flavas*, *Moraxella catarrhalis*, *Bacillus subtilis* ATCC, *Neisseria sicca* and *Candida albicans* CBS 5763.
[e] MIC value for *Escherichia coli* ATCC, MIC values for *Bacillus subtilis* ATCC ≥ 0.25 μg/ml and for *Staphylococcus aureus* ≥ 0.25 μg/ml.

(*Tillandsia usneoides*, L.) (Djerassi & McCrindle, 1962).

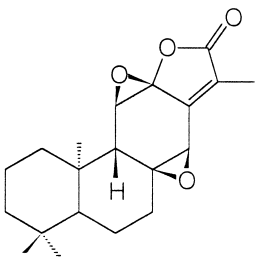
Compound **1** was assigned the molecular formula $C_{20}H_{28}O_3$ by HR-EIMS. It gave a purple color with anisaldehyde-sulfuric acid reagent on a TLC plate upon heating. The UV and IR spectra showed absorptions at $\lambda_{max}$ 276 nm (α,β,γ′,δ′-unsaturated-γ-lactone), $\nu_{max}$ 3328 and 1169 (hydroxyl group), 1710 (lactone C=O) and 1664 and 1600 $cm^{-1}$ (C=C). The $^1$H- and $^{13}$C-NMR spectra and the DEPT data showed signals for three tertiary methyl groups [$\delta_H$ 0.72, 0.86, and 0.92 (each 3H, *s*), $\delta_C$ 14.4, 22.0 and 33.5 (each *q*)], a vinyl methyl group [$\delta_H$ 1.83 (*s*); $\delta_C$ 8.1 (*q*)], a hemiacetal carbon [$\delta_H$ 3.17 (1H, *br s*, $D_2O$ exchangeable); $\delta_C$ 102.4 (*s*)], a >C=C–CH=C group [$\delta_H$ 6.19 (1H, *dd*, *J* = 2.0 and 2.0 Hz); $\delta_C$ 113.4 (*d*), 116.3, 154.2 and 154.4 (each *s*)] and a lactone carbonyl [$\delta_C$ 173.1 (*s*)], in addition to six methylenes, two methines and two quarternary carbons. Except for the presence of a hemiketal and one more methylene group, and the absence of a secondary hydroxyl group and an oxymethine group γ to the α,β-unsaturated-γ-lactone, the $^1$H- and $^{13}$C-NMR data of **1** were similar to those of *ent*-11α-hydroxyabieta-8(14),13(15)-dien-16,12α-olide (**7**) (Lal et al., 1990; Uemura & Hirata, 1972). In the COLOC experiment, $^3J$ correlations were observed for H-9 (with C-12) and H-14 (with C-12), indicative of **1** having the structure of 12-hydroxyabieta-8(14),13(15)-dien-16,12-olide as shown. The stereochemistry was examined by employing a selective NOE difference ex-



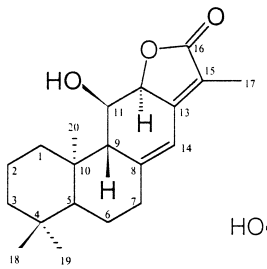
**1**



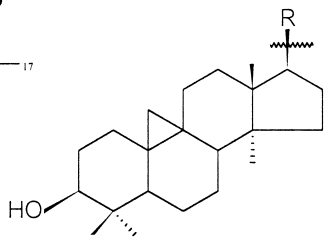
**2** R = $CH_3$
**8** R = $CH_2OH$

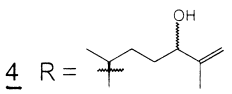


**3**

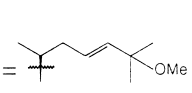


**7**

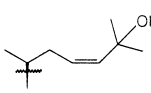




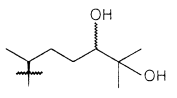
**4** R =



**6** R =



**5** R =



**9** R =

periment. Irradiation at the C-20 methyl protons at $\delta$ 0.72 showed NOE enhancements for Me-19 (2.8%), H-2α (3.3%), H-11α (3.7%) and H-14 (0.5%), as well as H-7β (2.0%) and H-9β (1.7%) by irradiation of the C-12 hydroxy proton, indicating Me-20 and the hydroxyl group of C-12 in compound **1** to have α (axial) and β (axial) orientations, respectively, with respect to ring C. In compound **7** bearing a 11β-hydroxy group, the H-12 adopts an α (axial) orientation (Lal et al., 1990). Thus, the structure of compound **1** was established as *ent*-12-hydroxy-12[*R*]-abieta-8(14),13(15)-dien-16,12-olide.

Due to the scarcity of pure samples, only some compounds isolated from the chloroform extract were further tested for antimicrobial activity against 21 strains of bacteria and yeasts, comprising 5 gram positive bacteria (*Bacillus cereus*, *B. subtilis* ATCC, *Micrococcus flavas*, *Staphylococcus aureus*, *S. epidermidis*), 14 gram negative bacteria (*Acinetobacter calcoaceticus*, *Aeromonas hydrophila*, *Moraxella catarrhalis*, *M. phenylpyruvica*, *Escherichia coli* ATCC, *Klebsiella pneumoniae*, *Neisseria mucusa*, *N. sicca*, *Providencia rettgeri*, *Pseudomonas aeruginosa* ATCC, *Salmonella choleraesuis*, *Serratia rubidiae*, *Shigella dysenteriae* and *Vibrio cholerae*) and two yeasts (*Candida albicans* CBS 5763 and *C. tropicalis*). Results are shown in Table 1.

Among the test compounds (see Table 1), the known *ent*-11α-hydroxyabieta- 8(14),13(15)-dien-16,12α-olide (**7**) showed moderate to strong growth inhibition against *B. cereus*, *B. subtilis*, *M. flavas*, *M. catarrhalis*, *N. sicca* and *C. albicans* CBS 5763 at 12.5 μg/ml concentration. Jolkinolide A (**2**) also moderately inhibited the growth of *M. catarrhalis* at 50 μg/ml concentration.

## 3. Experimental

### 3.1. General

Mps uncorrected; $^{1}$H- and $^{13}$C-NMR spectra were acquired in $CDCl_3$ and $CD_3OD$ using spectrometers at 400 and 100 MHz, respectively. The magnitude of the delay for optimizing one-bond correlation was 3.45 ms, and the evolution delay for long range coupling COLOC was set to 25 ms ($J$ = 20 Hz).

### 3.2. Plant material

Tubers of *E. sessiliflora* Roxb. were collected during Summer 1993 from Ratchaburi Province and identified by Assoc. Prof. Dr. Nijsiri Ruangrungsi, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330. A voucher specimen (SSES/1993) is deposited at the Department of Chemistry, Faculty of Science, Ramkhamhaeng University.

### 3.3. Extraction and isolation

The milled dry tubers of *E. sessiliflora* Roxb. (2.98 kg) were repeatedly macerated in methanol (10 days), and the methanolic solution was subsequently filtered and concentrated to a dark brown mass. The concentrated residue was partitioned between hexane and aqueous methanol; the hexane solubles were evaporated to dryness (8.13 g).The remaining aqueous methanolic layer was further partitioned with chloroform to give following solvent removal, a chloroform (33.7 g) and methanol (89.3 g) extract, respectively.

Only the chloroform extract showed antimicrobial activity on the assay using the agar dilution method (Baron, Peterson & Finegold, 1994). Silica gel CC of this extract eluted with hexane–chloroform to chloroform–methanol mixtures of increasing polarity (each fraction was monitored by TLC) led to five fractions. Fraction 1 was further subjected to silica gel CC (hexane–EtOAc, 4:1) to yield **2** and **3**. Fraction 2 was resubjected to silica gel CC (hexane–chloroform, 7:3) to yield a mixture of β-sitosterol and stigmasterol, **4**, **5** and **6**. Fraction 3 was rechromatographed (silica gel, hexane–chloroform, 1:3) to obtain two fractions. The less polar fraction was further chromatographed (silica gel, hexane–$CHCl_3$, 1:3) to yield **7**, and the more polar fraction (hexane–EtOAc, 99:1 to 7:3) afforded **1**, **8** and **9**. Fractions 4 and 5 were purified by silica gel CC using hexane–chloroform (15:85) and chloroform–MeOH (8:2) to yield a mixture of 3-β-D-glucopyranosides of sitosterol and stigmasterol and 3,3′,4-tri-*O*-methylellagic acid-4′-*O*-β-D-glucopyranoside, respectively.

Jolkinolide A (**2**), colorless needles (46.7 mg), mp 232°C (chloroform/methanol)(lit. mp 220°C Lal et al., 1990; Uemura & Hirata, 1972); jolkinolide B (**3**), colorless needles (16.6 mg), mp 223°C (chloroform/methanol) (lit. mp 220°C Uemura & Hirata, 1972); a mixture of β-sitosterol and stigmasterol, plates (101.4 mg); cycloart-25-en-3β,24-diol (**4**), colorless crystalline solid (4.2 mg), mp 153°C (hexane/chloroform); cycloart-23Z-en-3β,25-diol (**5**), colorless needles (8.4 mg), mp 185–186°C (hexane/chloroform); *ent*-11α-hydroxyabieta-8(14),13(15)-dien-16,12α-olide (**7**), prisms (31.4 mg), mp 195–196°C (chloroform/methanol) (lit. mp 198–199°C Lal et al., 1990); caudicifolin (**8**), colorless plates (5.4 mg), mp 186–187°C (hexane/chloroform) (lit. mp 184°C Satti et al., 1986); cycloartan-3β,24,25-triol (**9**), crystalline solid (4.7 mg), mp 154–155°C (chloroform/methanol) (lit. mp 154–156°C Teresa et al., 1987); a mixture of 3β-D-glucopyranosides of sitosterol and stigmasterol, powder (46.0 mg), 3,3′,4-tri-*O*-methylellagic acid-4′-*O*-β-D-glucopyranoside, powder

(33.2 mg.), mp 249–250°C, tetraacetate derivative, mp 263°C (lit. tetraacetate derivative mp 242–244°C Do Khac et al., 1990).

### 3.4. ent-12-Hydroxy-12[R]-abieta-8(14),13(15)-dien-16,12-olide (*1*)

Colorless needles, mp 192–193°C (hexane/chloroform), 14.8 mg, $[\alpha]_D^{24}$ −94.58° ($CHCl_3$:*c* 0.295); IR $\nu_{max}$ (KBr) $cm^{-1}$: 3328, 2940, 1710, 1664, 1600, 1462, 1169, 964, 944, 914, 880; UV $\lambda_{max}$ nm (log $\varepsilon$): 276 (4.076); $^1$H-NMR spectral data ($CDCl_3$): $\delta_H$ 0.72 (3H, *s*, H-20), 0.86 (3H, *s*, H-19), 0.90 (3H, *s*, H-18), 1.10 (1H, *m*, H-1), 1.20 (1H, *dd*, *J* = 12.5, 2.6 Hz, H-5), 1.21 (1H, *m*, H-3), 1.40 (1H, *m*, H-2), 1.43 (1H, *ddd*, *J* = 13.0, 12.8, 4.6 Hz, H-2′), 1.44 (1H, *m*, H-6), 1.45 (1H, *m*, H-3′), 1.57 (1H, *m*, H-1′), 1.58 (1H, *dd*, *J* = 13.0, 9.7 Hz, H-11ax), 1.74 (1H, *m*, H-6′), 1.83 (3H, *s*, H-17), 2.29 (1H, *dd*, *J* = 14.8, 7.4 Hz, H-9ax), 2.35 (1H, *dd*, *J* = 13.0, 6.0 Hz, H-7ax), 2.53 (1H, *ddd*, *J* = 13.6, 4.6, 3.0 Hz, H-7eq), 3.17 (1H, *br s*, OH-12), 6.19 (1H, *dd*, *J* = 2.0, 2.0 Hz, H-14); $^{13}$C-NMR spectral data ($CDCl_3$): $\delta_C$ 8.1 (*q*, C-17), 14.6 (*q*, C-20), 18.6 (*t*, C-2), 22.0 (*q*, C-19), 22.3 (*t*, C-6), 31.2 (*t*, C-11), 33.4 (*s*, C-4), 33.5(*q*, C-18), 36.0 (*t*, C-7), 38.9 (*t*, C-10), 39.0 (*t*, C-1), 41.7 (*t*, C-3), 51.4 (*d*, C-9), 54.0 (*d*, C-5), 102.4 (*s*, C-12), 113.4 (*d*, C- 14), 116.3 (*s*, C-15), 154.2 (*s*, C-13), 154.4 (*s*, C-8), 173.1 (*s*, C-16); EIMS 70 eV *m*/*z* (rel. int.): 316 ($[M]^+$, 5), 298 (13), 283 (7), 213 (7), 201 (9), 187 (9), 174 (26), 160 (82), 137 (57), 81 (55); HR-EIMS *m*/*z* 316.1948 $[M]^+$, $C_{20}H_{28}O_3$ requires 316.2038.

### 3.5. Antimicrobial assays (agar dilution method)

Twenty one strains of organisms were used for antimicrobial activity tests using the method of Baron et al. (1994). Results are shown in Table 1.

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