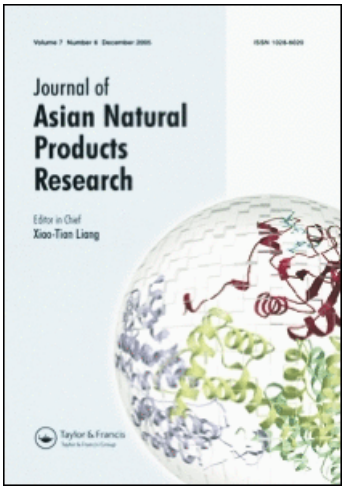

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A NEW SESQUITERPENE LACTONE FROM *TSOONGIODENDRON ODORUM* CHUN

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A new sesquiterpene lactone (**1**) was obtained from the cytotoxic fraction of 95% ethanol extract of root barks of *Tsoongiodendron odorum* Chun together with two known sesquiterpene lactones, costunolide (**2**) and parthenolide (**3**). The structure of **1** was elucidated as 5α, 6α, 7β, 10β–11α, 13-dihydro-4(15)-eudesmene-12, 6-olide on the basis of chemical and spectral evidence including X-ray diffraction analysis. Costunolide showed cytotoxic activity against human leukemia (HL-60) cell line. Parthenolide showed promising cytotoxic activities *in vitro* against HCT-8, Bel-7402, SKOV3, KB, HELA and EJ cell lines. Also, the cytotoxic ethyl acetate fraction of ethanol extract of the root barks from which three chemical components were isolated showed promising cytotoxic activities *in vitro* against KB, BGC-823, Bel-7402, HCT-8, HL-60 cell lines.

Keywords: *Tsoongiodendron odorum* Chun; Sesquiterpene lactones; Cytotoxic activity

INTRODUCTION

Tsoongiodendron odorum Chun (*Magnoliaceae*) is a large evergreen tree, only growing in the South of China. Now, it has become a special plant of China, being nearly extinct. Some other related plants of this family are important traditional Chinese medicines, which show antitumour activity, antibacteria activity and anti-ulcer activity *etc.* [1–3]. Up to now, there is no

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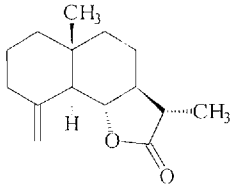
report on the chemical constituents of *T. odorum* in the literature except fatty acids analysis [4]. Furthermore, the whole plant has not previously been investigated for cytotoxic activity. It thus is apparent that the work on biological and chemical investigation of this plant is of interest. The ethyl acetate fraction of 95% ethanol extract of the root barks exhibited cytotoxic actvities against KB, BGC-823, Bel-7402, HL-60, HCT-8 cell lines tested in our screening program.

Our work led to the isolation of a new sesquiterpene lactone, 5α, 6α, 7β, 10β–11α, 13-dihydro-4(15)-eudesmene-12, 6-olide (**1**) together with two known sesquiterpene lactones, costunolide (**2**), parthenolide (**3**) as well as β-sitosterol.



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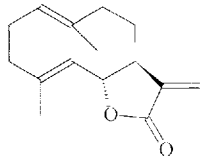

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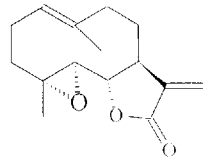

3

RESULTS AND DISCUSSION

Compound **1** was obtained as colorless plates. It showed a red spot with 5% H_2SO_4 ethanol followed by heating. The structure assignment of **1** was mainly based on X-ray analysis, which afforded the molecular weight 234.34 and molecular formula $C_{15}H_{22}O_2$. The ^1H NMR and ^{13}C NMR data were in accord with this structure. The EI-mass spectrum of **1** exhibited a molecular ion peak at m/z 234. According to the ^{13}C NMR spectrum, **1** included 15 carbons, and δ 179.5 was the signal of a typical lactonic carbonyl group. Furthermore, the data of ^1H NMR and ^{13}C NMR spectrum of **1** were completely consistent with the eudesmene type sesquiterpene lactone afforded by X-ray analysis. The ^1H NMR spectrum (500 MHz, $CDCl_3$) showed absence of the α-methylene group of the lactone ring for two allylically coupled protons were not observed, which usually appear at both δ 6.2 and δ 5.5 as doublets. Instead, there is a one-proton (doublet) at δ 4.91 coupling with one-proton (doublet) at δ 4.75 indicating the existence of an exocyclic methylene group. Two methyl signals appeared at δ 0.86 (10-Me, singlet) and at δ 1.22 (11-Me, doublet). In the ^{13}C NMR spectrum, only two olefinic carbon signals were observed at δ 144.7 (C-15) and δ 108.8 (C-4). See Table I.

TABLE I NMR chemical shifts assignment of compound **1** (500 MHz for ^{1}H and 125 MHz for ^{13}C, $CDCl_3$)

Position	δ_H	δ_C
1		41.8
2		22.8
3	2.00, *m*	41.2
4		108.8
5	1.799, *d* (8.5)	54.4
6	3.99, *dd* (10.5/8.5)	79.8
7	2.33, *m*	52.7
8	1.63, *m*	18.0
9	2.00, *m*	38.5
10		39.3
11	2.13, *d* (11)	35.9
12		179.5
13	1.22	12.5
14	0.86	23.5
15	4.95, *d*, *a* (3.5) 4.75, *d*, *b* (3.5)	144.7

Based on the above evidence as well as by comparison with data for the reference reported [5], compound **1** was determined as 5α, 6α, 7β, 10β–11α, 13-dihydro-4(15)-eudesmene-12,6-olide, which was a new compound.

For a perspective view of the structure of compound **1**, see Figure 1.

^{1}H-NMR and ^{13}C-NMR data for costunolide (**2**) were in accordance with those reported earlier [6]. DEPT, HMQC supported the structure. In addition, the $[M^+]$ at 232 and elemental analysis (C: 77.36%, H: 9.00%) gave powerful evidence for the molecular formula $C_{15}H_{20}O_2$.

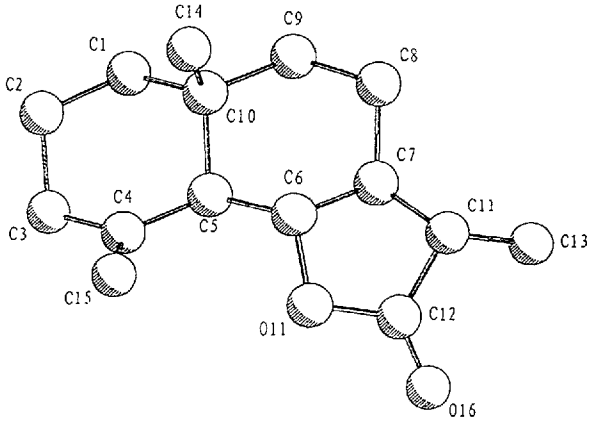

FIGURE 1 A perspective view of the structure compound **1**.

TABLE II Cytotoxic activities of extract and compounds **2**, **3**

Cytotoxic extract	*IC_{50} on cell lines tested (μg/ml)*							
and compounds	*KB*	*BGC-823*	*Bel-7402*	*HL-60*	*HCT-8*	*$SKOV_3$*	*HELA*	*EJ*
ethanol acetate extract	5.26	5.14	5.26	5.88	61.28			
Costunolide			–	10	–	–	–	–
Parthenolide	6.14	–	9.16	–	5.69	5.68	7.44	8.98

Both the ^{1}H-NMR and ^{13}C-NMR spectral data of compound **3** fit completely with those published for parthenolide [7], the [M^+] at 248 and DEPT supported the above result.

Activities of the Extract and Compounds 1, 2

The results of cytotoxicity evaluation of the ethyl acetate soluble fraction which was obtained from 95% ethanol extract of root barks of *T. odorum* measured by SRB assay, are summarized in Table II. The results from this screening assay gave evidence of the presence of effective cytotoxic constituents in the root barks of *T. odorum*. Thus, a separation of the active fraction was undertaken. Two known cytotoxic constituents, costunolide (**2**), parthenolide (**3**) and (**1**) were isolated successively from *T. odorum* for the first time.

The cytotoxicity of costunolide [8] has been reported previously. It is known for its cytotoxity against carcinoma of the nasopharynx. Parthenolide demonstrated significant activity against human laryngeal epidermoil carcinoma ($ED_{50} = 0.76$) [9] and the KB cell culture system ($ED_{50} = 0.45$) [8].

In our cytotoxic activity assay, costunolide (2) showed cytotoxic activity against human leukemia (HL-60) to a certain extent. Parthenolide showed significant activities against HCT-8, SKOV3, KB, HELA and EJ cell lines. The results of cytotoxicity evaluation of costunolide (by MTT assay) and parthenolide (by SRB assay) are summarized in Table II as well.

EXPERIMENTAL SECTION

General Experimental Procedures

Melting point was dertermined on a XT4A micromelting point apparatus and are uncorrected. EI-MS were obtained on AEI-MS-50 spectrometer.

NMR spectra were taken on INOVA-500 spectromer. Analytical thin-layer chromatography (TLC) was performed on Merck precoated silica gel 60 F_{254} plates and compounds were visualized by spraying with 5% H_2SO_4 ethanol followed by heating.

Plant Material

Dried root barks of *T. odorum* (5 kg) were provided by Huanan Botanical Gardens in Guangzhou City, Guangdong Provinece, China and was authenticated by Prof. Qingwen Zeng of Huanan Botanical Gardens. A voucher specimen of plant material has been deposited in the Department of Natural Medicines, School of Pharmaceutical Sciences. Peking University.

Extraction and Isolation

Dried root barks of *T. odorum* (5 kg) were extracted with 95% ethanol. The ethanol solution was concentrated and dried to afford ethanol extract (0.285 kg). The ethanol extract was partitioned between water and ethyl cetate to give ethyl acetate extract (EAE) (120.5 g).

The EAE (120.5 g) was subjected to column chromatography on silica gel and eluted with a mixture solvent of petrol and acetone (10 : 1 – 1 : 1). Three fractions (I – III) were collected according to TLC analysis.

Fraction I (approx. 3 g) was rechromatographed on a silica gel column, which was eluted with a mixture solvent of petrol and acetone (7 : 1) to give **1** (20 mg). Fraction II (approx. 10 g) was rechromatographed on a silica gel column, which was eluted with a mixture solvent of petrol and acetone (7 : 1) to afford **2** (120 mg). Fraction III (approx. 4 g) was rechromatographed on a silica column which was eluted with mixture solvent of petrol and acetone (4 : 1) to afford **3** (30 mg).

Compound **1** was obtained as colorless plates from acetone. M.p. 138 – 139°C. EI-MS m/z (rel. abunds): 234 (77.6) $[M^+]$, 219 (61.3) $[M\text{-}CH3]^+$, 206 (6.6) $[M\text{-}CO]^+$, 191 (11.7), 175 (38.3), 165 (base peak); for data of ^1H NMR and ^{13}C NMR, see Table I.

Single Crystal X-ray Analysis of Compound 1

Colourless single crystals were obtained from the solution of the compound in acetone. Crystal data: $C_{15}H_{22}O_2$. A crystal (dimention 0.40 × 0.32 × 0.10 mm) was used for collecting intensity data on a DIP-2030 K diffractometer. The compound crystallized in the orthohombic crystal form

in space group $P2_1$, $a = 7.438(1)$, $b = 7.941(1)$, $c = 23.323(3)$ Å, $V = 1377.6(s)$ Å^3; $Z = 4$; Dcalc = 1.135 g/cm^3; molecular weight 234.34, ω-scans by the rotation range 0–180°; the interval 5°; the distance between the crystal and the IP was 100 mm. A total of 1409 unique reflections were collected, of which 1396 observed reflections having ($|F|^2 \geq 8\sigma|F|^2$) were used. The structure was solved by direct methods by using SHELXS-86 program and refined by block-matrix least-squares techniques for all nonhydrogen atoms. The final R factor was 0.064.

Costunolide (**2**) was crystallized from MeOH as needles, m.p. 103–105°C, $[\alpha]_D$ +121(lit [10]. M.p. 106–107, $[\alpha]_D$ +128), m/z 232; NMR ($CDCl_3$): δ1.43 (*d*, 3 H, $J = 1$ Hz, C_{10}—Me or C_4—Me), 1.69 (*d*, 3 H, $J = 1$ Hz, C_4—Me or C_{10}—Me), 4.54 (*t*, 1 H, $J = 10$ Hz, C_6—H), 4.5–5.0 (br, 1 H, C_1—H), 4.79 (*m*, 1 H, C_4—H), 5.52 (*d*, 1 H, $J = 3$ Hz, C_{13}—H_a) and 6.24 ppm (*d*, 1 H, $J = 3$ Hz, C_{13}—H_b). This compound was identical with an authentic sample of costunolide.

Parthenolide (**3**) was crystallized from $CHCl_3$ MeOH as colorless needles, m.p. 114–115°C; NMR ($CDCl_3$): δ 1.30 (*s*, 3 H, C_4—Me), 1.72 (br, *s*, 3 H, C_{10}—Me), 2.78 (*d*, 1 H, $J = 8.5$ Hz, C_5—H), 3.91 (*t*, 1 H, $J = 8.5$ Hz, C_6—H), 5.27 (br, 1 H, C_1—H), 5.62 (*d*, 1 H, $J = 3$ Hz, C_{13}—H_a) and 6.31 ppm (*d*, 1 H, $J = 3$ Hz, C_{13}—H_b). This compound was identical with an authentic sample of parthenolide.

Assay Methods for Cytotoxic Activity

Human nasal epidermoil carcinoma (KB), human gastric carcinoma (BGC-823), human liver carcinoma (Bel-7402), human colon tumor (HCT-8), human cervix (of womb) carcinoma (HELA), human urinary carcinoma (EJ) and human ovary carcinoma (SKOV3) were used for the cytotoxic evaluations. These evalutions were used for the cytotoxic evaluations. These evalutions were carried out according to the sulforhodamine B (SRB) assay described previously [11]. This colorimetric assay estimates cell number indirectly by stainning total cellular protein with the dye SRB. Cytotoxicity against human leukemia (HL-60) cell lines were carried out according to the MTT (3-[4,5-di-methyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay as described in the Ref. [12].

Acknowledgements

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