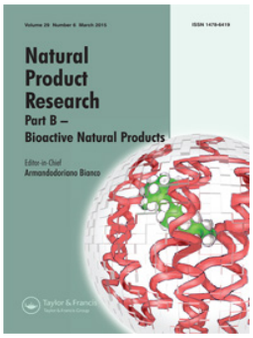

Natural Product Research
Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: http://www.tandfonline.com/loi/gnpl20

A new daphnane diterpenoid from Excoecaria venenata with inhibitory effect on human leukaemia HL-60 cells

De-Sheng Ning, Li-Yan Peng, Shi-Hong Lv, Dian-Peng Li & Zheng-Hong Pan

To cite this article: De-Sheng Ning, Li-Yan Peng, Shi-Hong Lv, Dian-Peng Li & Zheng-Hong Pan (2015) A new daphnane diterpenoid from Excoecaria venenata with inhibitory effect on human leukaemia HL-60 cells, Natural Product Research, 29:6, 524-528, DOI: 10.1080/14786419.2014.953498

To link to this article: http://dx.doi.org/10.1080/14786419.2014.953498

Natural Product Research, 2015
Vol. 29, No. 6, 524–528, http://dx.doi.org/10.1080/14786419.2014.953498

A new daphnane diterpenoid from *Excoecaria venenata* with inhibitory effect on human leukaemia HL-60 cells

De-Sheng Ning[a], Li-Yan Peng[b], Shi-Hong Lv[a], Dian-Peng Li[a] and Zheng-Hong Pan[a]*

[a]*Guangxi Key Laboratory of Functional Phytochemicals Research and Utilization, Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region and Chinese Academy of Sciences, Guilin 541006, P.R. China;* [b]*State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Science, Kunming 650204, P.R. China*

(*Received 23 June 2014; final version received 6 August 2014*)

A new daphnane diterpenoid, venenatin (**1**), along with six known compounds, was isolated from the aerial parts of *Excoecaria venenata*. The structure of **1** was elucidated on the basis of extensive spectroscopic analysis. Compound **1** exhibited growth inhibitory effect on human leukaemia HL-60 cell line with IC_{50} value of 28.10 μM.

Keywords: *Excoecaria venenata*; daphnane diterpenoid; HL-60

1. Introduction

Excoecaria venenata S. Lee and F.N. Wei (Euphorbiaceae) is a shrub endemic to China, mainly distributed in the Karst region of Southwest China, and has been used as a folk medicine to cure psoriasis and chronic eczema (Li & Wei 1982). Previous studies on this species demonstrated the presence of alkaloid, phenolic acid, triterpenoid, steroid and volatile components (Liu et al. 1992; Fu et al. 2006; Lu et al. 2006). In our search for structurally diverse and biologically interesting natural product from Karst plants, a new daphnane diterpenoid, venenatin (**1**), along with six known compounds, has been isolated from the aerial parts of *E. venenata*. The known compounds were *ent*-13-*epi*-8,13-epoxy-2,3-secolabd-14-ene-2,3-dioic acid (**2**), baccatin (**3**), cerevisterol (**4**), *N*-benzoylphenylalaninyl-*N*-benzoylphenylalaninate (**5**), apocynol A (**6**) and glyceroyl monopalmitate (**7**) (Takaishi et al. 1991; Fu et al. 1997; Konish et al. 1998; Murakami et al. 2001; Catalán et al. 2003; Wang et al. 2003). Compounds **1** and **2** were the first report of diterpenoids isolated from this plant and **1** was the second daphnane-type diterpenoid obtained from the genus *Excoecaria* (Wiriyachitra et al. 1985). The other known compounds were also reported from *E. venenata* for the first time. Moreover, venenatin (**1**) exhibited growth inhibitory effect on human leukaemia HL-60 cell line with IC_{50} value of 28.10 μM.

2. Results and discussion

Compound **1**, obtained as a white amorphous powder, had a molecular formula of $C_{20}H_{28}O_8$ as determined by the positive HR-ESI-MS ion at *m/z* 419.1678 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_8Na$, 419.1676) with seven degrees of unsaturation. The IR absorptions spectrum revealed the presence of hydroxyl (3424 cm^{-1}), carboxyl (1695 cm^{-1}) and double bond (1629 cm^{-1}). Analysis of the ^{13}C NMR and DEPT spectra displayed the presence of 20 carbon resonances, including 3 methyls, 3 methylenes (1 oxygenated), 7 methines (3 oxygenated and 1 olefinic), and 7 quaternary carbons (4 oxygenated and 2 olefinic). In addition, two tertiary methyls at δ_H 1.81

*Corresponding author. Email: pan7260@126.com

(s, 3H) and 1.73 (s, 3H), one secondary methyl at δ_H 0.95 (d, $J = 7.0$, 3H) and a terminal double bond at δ_H 5.09 (br s, 1H) and 5.03 (br s, 1H) could be distinguished in the ^{1}H NMR spectrum. The aforementioned evidence revealed that compound **1** possessed the characteristic A, B and C rings of a daphnane diterpenoid skeleton. The above-mentioned analysis accounted for six out of seven degrees of unsaturation, indicating the existence of an oxirane ring in compound **1**. This conclusion was supported by the upfield shifted C-6 (δ_C 64.0) and C-7 (δ_C 65.4) signals. The ^{1}H and ^{13}C NMR spectra were similar to those of wikstroelide M (Abe et al. 1998), a known daphnane diterpenoid isolated from *Wikstroemia retusa*. The main differences were the absence of a tetradecadienoate ester moiety attached at C-14 in wikstroelide M, and H-14 at δ_H 5.67 in wikstroelide M was found at δ_H 4.06 in compound **1**, suggesting that the tetradecadienoate ester was replaced by an OH in **1**. This was consistent with the molecular formula $C_{20}H_{28}O_8$. The correlations in the ^{1}H–^{1}H COSY and HMBC spectra further confirmed the planar structure of **1** (see Supplementary material, Figure S1). The relative configuration of **1** was elucidated by NOESY spectrum (see Supplementary material, Figure S1). The NOESY correlations of H-7/H-8, H-14 and H_2-20, H-8/H-11, H-14 and H-16, and H-16/H-11 and H-14 suggested that these protons were β-oriented. Although H-5 and H-10 were almost overlapped in the ^{1}H NMR spectrum, H-5 and H-10 displayed no any correlation with the above-mentioned protons in the NOESY spectrum, indicating an α-orientation of two protons. From a biogenetic point view, the A/B and B/C ring junctions of all daphnanes isolated so far are trans-configuration, so the relative configurations of 4-OH and 9-OH were also suggested to be β-oriented. On the basis of these analysis and referring to reported daphnane diterpenoids (Wang et al. 2013), compound **1** was assigned, as shown in Figure 1, the name venenatin.

The known compounds were determined as *ent*-13-*epi*-8,13-epoxy-2,3-secolabd-14-ene-2,3-dioic acid (**2**), baccatin (**3**), cerevisterol (**4**), *N*-benzoylphenylalaninyl-*N*-benzoylphenylalaninate (**5**), apocynol A (**6**), and glyceroyl monopalmitate (**7**), respectively, by direct comparison of their spectral data with those in the literature (Takaishi et al. 1991; Fu et al. 1997; Konish et al. 1998; Murakami et al. 2001; Catalán et al. 2003; Wang et al. 2003). All isolated compounds were reported from *E. venenata* for the first time. Compounds **1** and **2** were the first report of diterpenoid isolated from this plant and **1** was the second daphnane-type diterpenoid obtained from the genus *Excoecaria* (Wiriyachitra et al. 1985).

Compounds **1**–**7** were evaluated for their cytotoxic activities against human leukaemia HL-60 cell line using cisplatin (DDP) as a positive control ($IC_{50} = 2.03$ μM) (Mosmann 1983). The new compound, venenatin (**1**), exhibited growth inhibitory effect on human leukaemia HL-60 cell line with IC_{50} value of 28.10 μM, while the other compounds were inactive.

3. Experimental

3.1. *General experimental procedures*

Optical rotations were measured with a Perkin-Elmer 341 polarimeter (Perkin-Elmer, Waltham, MA, USA). UV data were recorded using a Shimadzu UV-2401A spectrophotometer (Shimadzu Corp., Kyoto, Japan). IR spectra were obtained by a Nicolet 6700 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) with KBr pellets. 1D and 2D NMR spectra were run on Bruker DRX-500 spectrometer (Bruker Co., Ettlingen, Germany) with TMS as internal standard. Mass spectra were recorded on an API QSTAR Pulsar 1 spectrometer (Applied Biosystems, Foster City, CA, USA). Column chromatography (CC) was performed using silica gel (100–200 and 200–300 mesh, Qingdao Marine Chemical Co. Ltd, Qingdao, China), RP-18 gel (40–63 μm, Merck, Darmstadt, Germany), MCI gel (75–150 μm; Mitsubishi Chemical Corporation, Tokyo, Japan) and Sephadex LH-20 (Amersham Pharmacia Biotech, Uppsala, Sweden). Fractions were monitored by TLC (GF254, Qingdao Marine Chemical Co. Ltd), and spots were visualised by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH. All solvents were distilled prior to use.

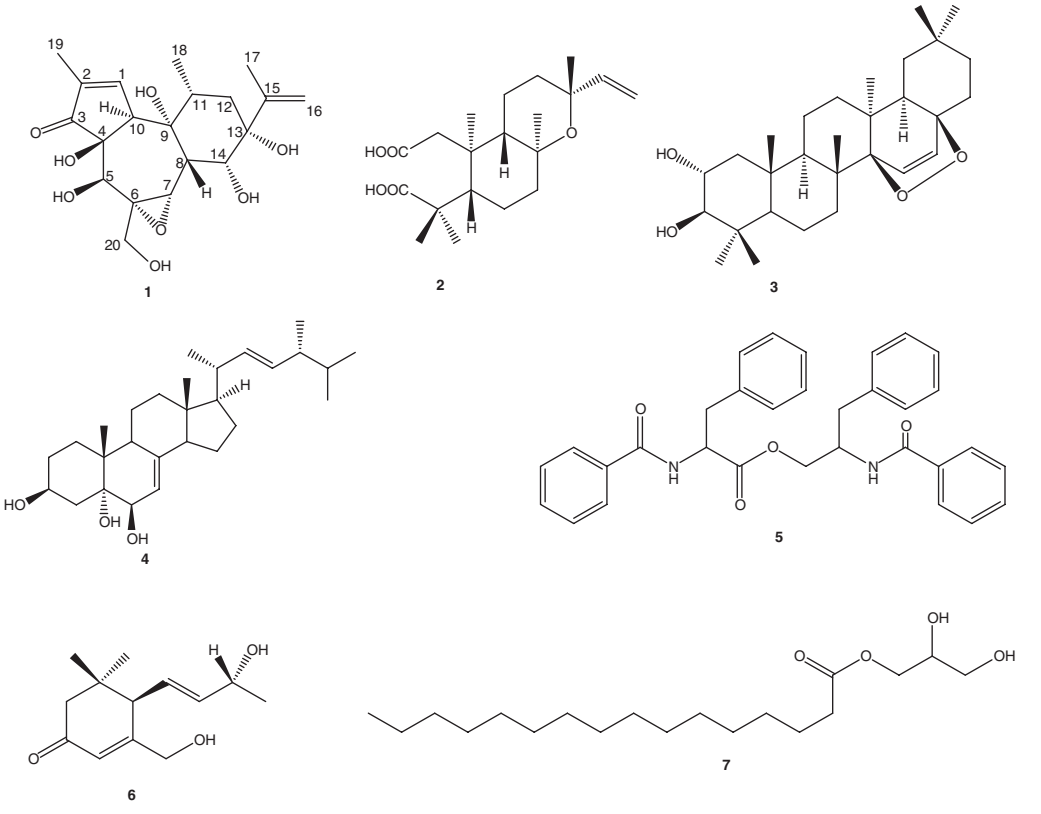

Figure 1. Structure of compounds **1**–**7**.

3.2. *Plant material*

The aerial parts of *E. venenata* S. Lee and F.N. Wei were collected in September 2011 from Longzhou County, Guangxi Province, People's Republic of China, and identified by Prof. Fa-Nan Wei of Guangxi Institute of Botany. The voucher specimen (CTM201102) has been deposited at the Guangxi Key Laboratory of Functional Phytochemicals Research and Utilization.

3.3. *Extraction and isolation*

The air-dried aerial parts of *E. venenata* (13 kg) were extracted with EtOH (3 × 50 L) for 24 h every time at room temperature. The extract was evaporated to obtain a residue, which was suspended in H_2O (2 L) and then partitioned with EtOAc (3 × 1.5 L). The EtOAc extract (386 g) was decolourised on MCI gel (eluted with 90% EtOH) and then subjected to a silica gel (100–200 mesh) CC eluted with gradient petroleum ether–Me_2CO (1:0 → 0:1) to obtained five fractions (A–E). Fraction B (80 g) was further subjected to a silica gel CC eluted with petroleum ether–EtOAc (1:0 → 0:1) to provide four subfractions (B_1–B_4). Subfraction B_2 (26 g) was chromatographed over repeated silica gel CC combined with Sephadex LH-20 eluted with $CHCl_3$–MeOH (1:1) to afford **4** (28 mg) and **6** (15 mg). Subfraction B_3 was applied to an RP-18 gel eluted with MeOH–H_2O (1:1 → 1:0) followed by repeated silica gel CC (petroleum ether–EtOAc, 8:2) to afford **3** (44 mg) and **7** (35 mg). Compound **5** (45 g) was obtained by recrystallisation in Me_2CO from the subfraction B_4. Fraction C was chromatographed over silica gel CC eluted with petroleum ether–Me_2CO (8:2), and then separated by semi-preparative HPLC (75% MeOH–H_2O) to yield **2** (8 mg) and **1** (7 mg).

3.3.1. *Venenatin (**1**)*

White amorphous powder; $[\alpha]_D^{25.1} = -23.2$ ($c = 0.09$, MeOH); UV (MeOH) λ_{max} (log ε) 200 (3.30), 247 (3.42); IR (KBr cm^{-1}) ν_{max}: 3424, 2926, 1695, 1629, 1384, 1111, 1032 1012; HR-ESI-MS *m/z*: 419.1678 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_8Na$, 419.1676). ^1H NMR data (CD_3OD; 500 MHz), δ: 7.57 (1H, s, H-1), 4.07 (1H, s, H-5), 3.29 (1H, s, H-7), 3.24 (1H, s, H-8), 4.06 (1H, overlap, H-10), 2.16 (1H, m, H-11), 1.94 (1H, dd, $J = 13.0$, 14.0 Hz, H-12a), 1.71 (1H, overlap, H-12b), 4.06 (1H, overlap, H-14), 5.09 (1H, s, H-16a), 5.03 (1H, s, H-16b), 1.81 (3H, s, H_3-17), 0.95 (3H, d, $J = 7.0$ Hz, H_3-18), 1.73 (3H, s, H_3-19), 3.98 (1H, d, $J = 12.5$ Hz, H-20a), 3.58 (1H, d, $J = 12.5$ Hz, H-20b). ^{13}C NMR data (CD_3OD; 125 MHz), δ: 162.2 (C-1), 135.5 (C-2), 209.9 (C-3), 74.6 (C-4), 70.5 (C-5), 64.0 (C-6), 65.4 (C-7), 39.1 (C-8), 79.8 (C-9), 51.0 (C-10), 39.5 (C-11), 39.1 (C-12), 75.4 (C-13), 79.4 (C-14), 147.2 (C-15), 114.7 (C-16), 19.3 (C-17), 18.3 (C-18), 9.9 (C-19), 65.0 (C-20).

3.4. *Cytotoxic activity*

Compounds **1**–**7** were examined for their cytotoxic activity against the human leukaemia HL-60 cell line using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method (Mosmann 1983). Cells were plated in 96-well plates 12 h in the presence of different concentrations of compounds from DMSO-diluted stock solutions. After 48 h, 20 μL of MTT solution were added to each well, which were incubated for a further 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 (Reed & Muench 1938).

4. Conclusion

In summary, seven compounds were isolated from the aerial part of *Excoecaria venenata*, including a new daphnane diterpenoid named as venenatin (**1**). It exhibited growth inhibitory effect on human leukaemia HL-60 cell line with IC_{50} value of 28.10 μM.

Supplementary material

Supplementary material relating to this article is available online, alongside structure NMR spectra of all isolated compounds (Figures S2–S23) and Figure S1.

Funding

This work was financially supported by the National Natural Science Foundation of China [grant number 21102023] and the Funds of Guangxi Key Laboratory of Functional Phytochemicals Research and Utilization [grant number ZRJJ2012-8], [grant number ZRJJ2013-8].

References

Abe F, Iwase Y, Yamauchi T, Kinjo K, Yaga S, Ishii M, Iwahana M. 1998. Minor daphnane-type diterpenoids from *Wikstroemia retusa*. Phytochemistry. 47:833–837.

Catalán CAN, de Heluani CS, Kotowicz C, Gedris TE, Herz W. 2003. A linear sesterterpene, two squalene derivatives and two peptide derivatives from *Croton hieronymi*. Phytochemistry. 64:625–629.

Fu HZ, Lou ZC, Yang XW, Cai SQ, Guo DA, Yu J, Yang XP, Li YS. 1997. Studies on the chemical constituents of Glandularstalk St. Paulswort (*Siegesbeckia pubescens*) (I). Chin Tradit Herb Drugs. 28:259–262.

Fu LN, Lu X, Liu CW, Wang H, Xiao CP. 2006. Two alkaloids from *Excoecaria venenata*. Guihaia. 26:221–222.

Konish T, Fujiwara Y, Konoshima T, Kiyosawa S. 1998. Five new labdane-type diterpenes from *Excoeccaria agallocha*. Chem Pharm Bull. 46:1393–1398.

Li SG, Wei FN. 1982. New plants on limestone hills from Guangxi. Guihaia. 2:129–133.

Liu SH, Xie YC, Cheng JY. 1992. Studied on the chemical constituents from *Excoecaria venenata*. Guihaia. 12:133–135.

Lu X, Liu CW, Fu LN, Xiao CP. 2006. GC/MS analysis of chemical constituents from volatiles in *Excoecaria venenata*. Guihaia. 26:107–109.

Mosmann T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 65:55–63.

Murakami T, Kishi A, Matsuda H, Hattori M, Yoshikawa M. 2001. Medicinal foodstuffs. XXIV. Chemical constituents of the processed leaves of *Apocynum venetum* L.: absolute stereostructures of apocynosides I and II. Chem Pharm Bull. 49:845–848.

Reed LJ, Muench H. 1938. A simple method of estimating fifty percent endpoints. Am J Hyg. 27:493–497.

Takaishi Y, Uda M, Ohashi T, Nakano K, Murakami K, Tomimatsu T. 1991. Glycosides of ergosterol derivatives from *Hericum erinacens*. Phytochemistry. 30:4117–4120.

Wang H, Zhang XF, Pan L, Yang SM, Ma YB, Luo XD. 2003. Chemical constituents from *Euphorbia wallichii*. Nat Prod Res Dev. 15:483–486.

Wang HP, Liu LP, Wang XY. 2013. 13C-NMR data of daphnane diterpenoids. Magn Reson Chem. 51:580–592.

Wiriyachitra P, Hajiwangoh H, Boonton P, Adolf W, Opferkuch HK, Hecker E. 1985. Investigations of medicinal plants of Euphorbiaceae and Thymelaeaceae occurring and used in Thailand. II. Cryptic irritants of the diterpene ester type from three *Excoecaria* species. Planta Med. 51:368–371.