Havanensin-type Limonoids from the Stem Bark of *Chisocheton pentandrus* (Meliaceae)

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Abstract. A new limonoid, belonging to the havanensin-type, called pentandricine E (**1**), alongside four other known types, i.e., trichilenone acetate (**2**), toonaciliatones C (**3**), 11α-acetoxyazadirone (**4**) and ceramicine I (**5**), were found in *Chisocheton pentandrus* stem bark. The structures of the isolated **1**-**5** were identified by NMR spectroscopy and HRTOFMS. Pentandricine E (**1**) displayed the highest cytotoxicity toward the MCF-7 breast cancer line with an IC_{50} value of 63.27 ± 0.21 μM.

Keywords: *Chisocheton pentandrus*; havanensin-type; MCF-7; pentandricine E.

1 Introduction

The *Chisocheton* genus, belonging to the Meliaceae plant family, is distributed in subtropical and tropical regions, and is recognized for its insecticidal limonoid content [1,2]. Moreover, previous investigation of *Chisocheton* species has revealed the presence of some limonoids [3-7], triterpenoids [8,9], sesquiterpenoids [10], and spermidine alkaloids [11]. Specifically, *C. pentandrus* is considered a higher plant, with growth restricted to the tropical rainforests of Indonesia and traditional application only in Indonesia for the treatment of wounds, diarrhea, fever, and stomach ulcers [12]. During our search for anticancer substances from *Chisocheton* species, we have described cytotoxic constituents from *Chisocheton* plants such as *C. paten* Blume [9], *C. macrophyllus* [13], *C. cumingianus* [14], and recently *C. pentandrus* [5,6]. In the previous investigations we also reported four havanensin-type limonoids, called pentandricine A-D, from the stem bark of *C. pentandrus* [5,6]. Further exploration of the non-polar fraction showed the presence of a new type of limonoid, called pentandricine E (**1**), alongside four known derivatives (**2**-**5**)

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(Figure 1). Here, the isolation and structural determination of the new limonoid and the four known ones are discussed as well as their cytotoxic effects.

2 Materials and Methods

2.1 General

The 1D and 2D NMR spectra were measured at room temperature on a JEOL JNM-ECZ500R/S1 spectrometer with $SiMe_4$ as the internal standard. The chemical shift values were referenced to $CDCl_3$ (δ_H 7.25, δ_C 77.0). NMR assignments were made using a combination of 1D and 2D techniques. HR-ESITOFMS data were recorded on a Waters Xevo HRTOFMS QTOF mass spectrometer. An ATAGO AP-300 polarimeter was used to obtain the optical rotation. UV and IR spectra were obtained on a TECAN Infinite M200 Pro and a Thermo Scientific Nicolet spectrophotometer, respectively. Column chromatography utilized silica gel (70-230 and 230-400 mesh, Merck). Silica gel plates GF_{254} (Merck) were used for thin-layer chromatography (TLC). Visualization: UV at 254 and 367 nm or *p*-dimethylaminobenzaldehyde and hydrochloric acid (1:1) in ethanol.

2.2 Plant Material

Stem bark of *C. pentandrus* was obtained from Bogor Botanical Garden, Indonesia in August 2017. Determination of the plant was performed by Mr. Harto (No. Bo-104) at Bogoriense Herbarium, Indonesia.

2.3 Extraction and isolation

Air-dried stem bark of *C. pentandrus* (1.6 kg) was powdered and then macerated with methanol (3 × 4 L) for three days, followed by concentration under vacuum. This process yielded a residue (340 g), from which about 300 g was dissolved in water (600 mL), followed by successive partitioning with *n*-hexane, EtOAc and *n*-BuOH. Furthermore, the residue was concentrated to produce 10.90, 25.18, and 228.63 g of residue extract. A cytotoxic test of the extracts was conducted to MCF-7 breast cancer cells, with *n*-hexane extract exhibiting the highest activity and thus further research focused on this extract. The *n*-hexane extract (11.0 g) was eluted with *n*-hexane:EtOAc (20:1 to 1:1) by SiO_2 column chromatography (CC) to yield eight fractions (A-H). Subsequently, fraction E (819.8 mg) was purified by CC and eluted with CH_2Cl_2-EtOAc (5% increase of polarity) to give yield five subfractions (E1-E5), while subfraction E3 (199.0 mg) was further submitted to CC on SiO_2 eluted with CH_2Cl_2-EtOAc (1:1) to yield **5** (6.2 mg). In addition, subfraction E4 (215.1 mg) was eluted with CH_2Cl_2-EtOAc (2.5%

increase of polarity) by SiO_2 CC to yield four subfractions (E4A-E4D). Therefore, subfraction E4B (30.0 mg) was eluted with CH_2Cl_2:EtOAc:HAc (6.0:3.5:0.5) by SiO_2 CC to yield **1** (6.0 mg). Also, subfraction E4C (215.1 mg) was eluted with CH_2Cl_2:EtOAc:MeOH (5.5:4:0.5) by SiO_2 CC to yield **2** (15.2 mg). Fraction G (780 mg) was eluted with $CHCl_3$-EtOAc (5% increase of polarity) by SiO_2 CC to yield seven subfractions (G1-G7). Subsequently, subfraction G3 (310 mg) was eluted with CH_2Cl_2:HAc:MeOH (8.5:0.5:1.5) by SiO_2 CC to produce four subfractions (G3A-G3D). Particularly, subfraction G3D (100 mg) was then eluted with CH_2Cl_2:HAc:MeOH (8.5:0.5:1.0) by SiO_2 CC to yield **3** (5.1 mg). In addition, subfraction G4 (130 mg) was eluted with CH_2Cl_2:HAc:MeOH (8.5:0.5:1.0) by SiO_2 CC to give **2** (8.2 mg) and **4** (5.2 mg).

Pentandricine E (**1**) is a colorless amorphous solid; $[\alpha]^{20}_D$ + 29^o (*c* 0.01, $CDCl_3$), UV (MeOH) λ_{max} 230 nm (log ε 4.2); IR (KBr) ν_{max} (cm^{-1}) 3540, 3450, 2862, 1720, 1690, 1457, 1387, 1247; ^1H and ^{13}C NMR data see Table 1; HR-TOFMS *m/z* 425.2021 $[M-H]^-$ (calculated $C_{26}H_{33}O_5$ *m/z* 426.2046).

2.4 Bioassay for Cytotoxic Activity

PrestoBlue™ Cell Viability Reagent is a commercially available and ready-to-use (Thermo Fisher, catalog number A13261) tool for MCF-7 viability assay. This assessment was carried out in line with the standard procedure, where cells in suspension are grown at 17,000 cells/well in a 96-well plate. The cells were washed with PBS and incubated with PrestoBlue reagent after 24 h of treatment with culture medium comprising DMSO (0.5% to 2%) alone and in combination with Cisplatin (200 ppm), or different doses of the active compound (ranging from 1000 ppm to 7.5 ppm). In addition, absorbance spectroscopy was used to detect modifications in cell viability, and records were obtained at 570 nm and 600 nm after incubating the MCF-7 cells with PrestoBlue reagent for 1 h. The background value was corrected using triplicate well sets containing the medium only (without cells) and added with PrestoBlue reagent in the experimental well. The data generated were analyzed and are presented here in the form of concentration level.

3 Results and Discussions

The air-dried powder of the stem bark of *C. pentandrus* was extracted with MeOH at room temperature to give a concentrated residue, followed by partitioning with *n*-hexane, EtOAc and *n*-BuOH, successively. Column chromatographic separations of the *n*-hexane extract yielded isolates **1**-**5** (Figure 1).

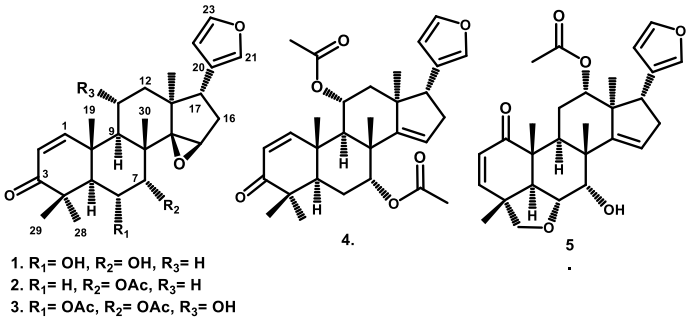

Figure 1 Structures of isolates **1**-**5**.

The molecular composition of **1** was assigned to be $C_{26}H_{34}O_5$ (ten degrees of unsaturation) according to HR-TOFMS *m/z* 425.2025 $[M-H]^-$ (calculated $C_{26}H_{33}O_5$ *m/z* 426.2046). The UV spectrum suggests the existence of an enone group at 230 nm (log ε 4.2) [15]. Moreover, the IR spectrum exhibited absorption typical of hydroxyl (3540 and 3450 cm^{-1}) and ketone (1690 cm^{-1}). The ^{1}H-NMR data of **1** (Table 1) exhibited the existence of five tertiary methyls at (δ_H 1.23, 1.21, 1.15, 1.14, and 0.93, each 3H), five sp^2 methine at [δ_H 7.10 (1H, d, *J* = 12.0 Hz), 5.80 (1H, d, *J* = 12.0 Hz), 7.08 (1H, s), 7.34 (1H, s) and 6.13 (1H, s)], and methine bearing oxygen at [δ_H 4.25 (1H, dd, *J* = 3.1, 15.0 Hz), 4.02 (1H, d, *J* = 3.1 Hz) and 3.41 (1H, s)]. Meanwhile, the ^{13}C NMR and HMQC spectra of compound **1** revealed the existence of one carbonyl (δ_C 204.8), five sp^2 methines (δ_C 157.8, 143.1, 139.6, 126.4, and 111.0), one sp^2 quaternary carbon (δ_C 123.7), one quaternary oxygenated carbon (δ_C 72.9), three sp^3 oxygenated methine carbons (δ_C 70.3, 73.7 and 57.3). These groups represent four degrees of unsaturation, while the other six require the existence of limonoids with an epoxy ring, resulting from the havanensin-type characteristics [5,6,16]. Comparison between NMR data of **1** and 6α-acetyloxy-14β,15β-epoxyazadirone isolated from *Entandrophrogma delevoyi* [17] showed a close relation between the structures of both compounds. The principal disparity was investigated in the loss of two acetyl groups at C-6 and C-7, and the appearance of two methines bearing oxygen at [δ_H 4.25 (1H, dd, *J* = 3.1, 15.0 Hz), 4.02 (1H, d, *J* = 3.1 Hz), δ_C 70.3 and 73.7)]. This suggests the tendency for **1** to be a deacetylated derivative of 6α-acetyloxy-14β,15β-epoxyazadirone. In addition, the location of a hydroxyl moiety was possibly clarified by conducting the 2D-NMR experiments shown in Figure 2. Particularly, the spectra produced with ^{1}H-^{1}H COSY support the existence of a limonoid skeleton, as the correlation of H-6/H-7 indicates attachment of the diol group at C-6 and C-7, while H-15/H-16 denotes the formation of an epoxide ring at C-14/C-15. Meanwhile, the HMBC spectrum indicates the association between the tertiary methyl and neighboring carbon. This allows the assignment of five singlet methyls at C-18, C-19, C-28, C-29 and C-30, respectively. Furthermore,

olefinic protons identified at δ_H 7.13 (H-1) and 5.85 (H-2) were correlated to the carbonyl at δ_C 204.8 (C-3). This phenomenon indicates the location of an α,β-unsaturated carbonyl at C-1, C-2 and C-3. A sp^2 proton at H-23 (δ_H 6.13) was correlated to C-22 (δ_C 111.0) and C-20 (δ_C 123.4) suggested the presence of a furan skeleton attached at C-17. Moreover, NOESY was used to determine the relative configuration of **1** and was performed by the existence of limonoids in the *Chisocheton* species [5,6,16]. Hence, the correlations established between CH_3-19, H-6 and H-7 indicate the existence of an α-oriented hydroxyl group at H-6 and H-7, whereas between CH_3-18 and H-15 there is a β-oriented epoxide group. The NOESY correlation examined between CH_3-30/H-17 shows the presence of an α-oriented γ-pyrone ring at C-17. Therefore, **1** was structurally determined as a new havenensin-type limonoid, called pentandricine E.

Table 1 NMR data of Compound **1.**

Carbon	δ_C (mult.)	δ_H (integral, mult., *J* = Hz)
1	157.8 (d)	7.10 (1H, d, 12.0)
2	126.4 (d)	5.80 (1H, d, 12.0)
3	204.8 (s)	-
4	40.6 (s)	-
5	48.5 (s)	2.50 (1H, d, 15.0)
6	70.3 (d)	4.25 (1H, dd, 3.1, 15.0)
7	73.7 (d)	6.13 (1H, d, 3.1)
8	45.3 (s)	-
9	39.1 (d)	2.58 (1H, m)
10	41.9 (s)	-
11	16.5 (t)	1.87 (1H, dd, 2,5, 4.6)
		1.60 (1H, d, 4.6)
12	31.7 (t)	1.57 (1H, dd, 2.5, 6.5)
		1.79 (1H, d, 6.5)
13	42.9 (s)	-
14	72.9 (s)	-
15	57.3 (d)	3.41 (1H, m)
16	32.2 (t)	2.58 (1H, m)
		2.12 (1H, d, 5.4)
17	39.4 (d)	2.62 (1H, m)
18	21.9 (q)	0.93 (3H, s)
19	29.3 (q)	1.14 (3H, s)
20	123.4 (s)	-
21	139.6 (d)	7.08 (1H, d, 3.0)
22	111.0 (d)	4.02 (1H, dd, 3.0, 6.2)
23	143.1 (d)	7.34 (1H, d, 6.2)
28	18.9 (q)	1.15 (3H, s)
29	20.3 (q)	1.21 (3H, s)
30	21.7 (q)	1.23 (3H, s)

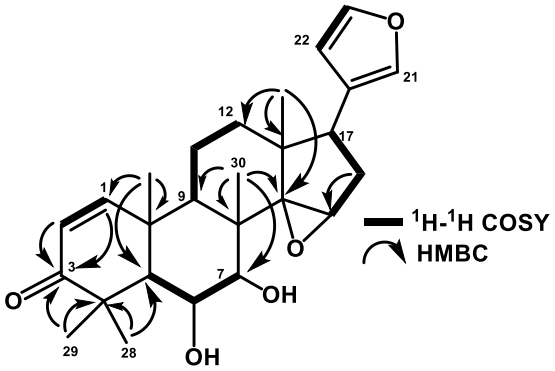

Figure 2 2D-NMR correlations of **1.**

The known limonoid compounds were identical to trichilenone acetate (**2**) [17], toonaciliatones C (**3**) [18], 11α-acetoxyazadirone (**4**) [19], and ceramicine I (**5**) [20] by comparison to previously reported spectroscopic data.

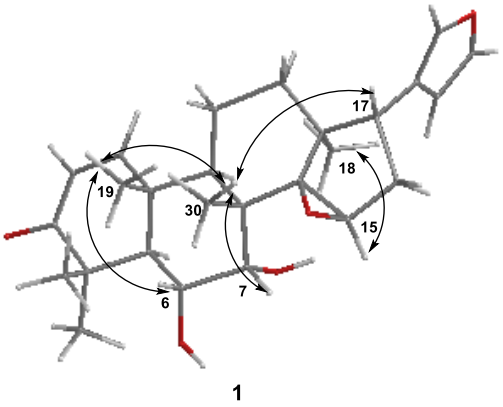

Figure 3 Key NOESY correlations for **1.**

The isolated limonoids **1-5** were also examined for their cytotoxic effects on the MCF-7 breast cancer cell line (Table 1) [20]. In addition, Cisplatin (IC_{50} 50.30 ± 0.02 µM) served as positive control [21]. Pentrandicine E (**1**) demonstrated the strongest cytotoxicity. This indicates a positive correlation between the increase of free -OH groups present at C-6 and C-7 and the cytotoxic activity in the limonoid structures. Also, the effect was enhanced compared with its reduced form by the oxidation of olefin into epoxide groups at C-15/C-16.

Table 2 Cytotoxicity of Compounds **1**-**5** (in *vitro*).

Compounds	IC_{50} (μM)
Pentandricine E (**1**)	63.27 ± 0.21
Trichilenone acetate (**2**)	101.98 ± 0.04
Toonaciliatones C (**3**)	209.90 ± 0.02
11α-acetoxyazadirone (**4**)	313.92 ± 0.02
Ceramicine I (**5**)	345.13 ± 0.08
Cisplatin*	50.30 ± 0.02

*Positive control

Conclusions

Five limonoids (**1**-**5**) from *Chisocheton pentandrus* stem bark were investigated, and their structures were identified by spectroscopic methods. Pentandricine E (**1**) displayed the highest effect with an IC_{50} value of 63.27 ± 0.21 μM.

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