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# A new limonoid from stem bark of *Chisocheton pentandrus* (Meliaceae)

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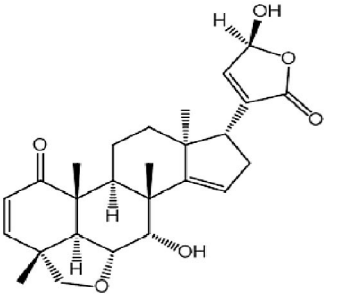
**ABSTRACT**

A new limonoid, pentandricine (**1**), along with three known limonoids, ceramicine B (**2**), 6-de(acetyloxy)-23-oxochisocheton (**3**), 6-de(acetyloxy)-23-oxo-7-*O*-deacetylchisocheton (**4**), have been isolated from the stembark of *Chisocheton pentandrus*. The chemical structures of the new compound were elucidated on the basis of spectroscopic evidence. All of the compounds were tested for their cytotoxic effects against MCF-7 breast cancer cells. Compounds **1**–**4** showed weak and no cytotoxicity against MCF-7 breast cancer cells with $IC_{50}$ values of 369.84, 150.86, 208.93 and 120.09 µM, respectively.

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*Chisocheton pentandrus*; limonoid; pentandricine; Meliaceae; MCF-7 breast cancer

## 1. Introduction

*Chisocheton* is genus belong to Meliaceae family, consist more than 50 plant species that are distributed mainly in India, Thailand, Malaysia and Indonesia (Heyne 1982; Yang et al. 2009). The genus *Chisocheton* was distributed in the tropical regions and widely known for

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its insecticidal limonoid constituents (Jain and Tripathi 1993; Roy and Saraf 2006). Previous phytochemical studies on *Chisocheton* species have yielded a number of interesting compounds, including limonoids (Connolly et al. 1979; Gunning et al. 1994), antifungal meliacin-type compounds (Bordoloi et al. 1993), dammarane-type triterpenoids, with an inhibitory effect on Epstein-Barr virus activation (Inada et al. 1993) and spermidine alkaloids (Tzouros et al. 2004).

In our continous search for novel cytotoxic constituents from Indonesian *Chisocheton* plants, we isolated a new limonoid, dysobinol, from the seed of *C. macrophyllus* (Nurlelasari et al. 2017), and a lanostane-type triterpenoid from the bark of *C. cumingianus* (Katja et al. 2016). In the further screening for novel cytotoxic compounds from Indonesia *Chisocheton* plants, we found that the methanol extract of *Chisocheton pentandrus* showed a significant cytotoxic activity against MCF-7 breast cancer cells. In this paper, we report the isolation and structural elucidation of a new limonoids, pentandricine (**1**) and known limonoid compounds **2**–**4**, along with their cytotoxic activity against MCF-7 breast cancer cells.

## 2. Results and discussion

The *n*-hexane extract of the bark of *C. pentandrus* was chromatographed over a vacuum-liquid chromatographed (VLC) column packed with silica gel 60 by gradient elution. The VLC fraction were repeatedly subjected to normal and reverse phase column chromatography and preparative TLC on silica gel $GF_{254}$ to afford compounds **1**–**4** (Figure 1).

Pentandricine (**1**) was obtained as a colourless amorphous solid. Its molecular composition was established to be $C_{26}H_{32}O_6$ from a combined analysis of the HR-ESI-TOFMS spectra (*m/z* 439.2025 $[M + H]^-$ and NMR data, thus requiring 11 degree of unsaturations. The UV spectrum showed an absorption maximun at 250 nm (log $\varepsilon$ 4.2), indicating the presence of a conjugated carbonyl group. The IR spectrum showed bands which were ascribed to hydroxyl ($\nu_{max}$ 3540 and 3450 $cm^{-1}$), an α,β-unsaturated carbonyl ($\nu_{max}$ 1690 $cm^{-1}$), a conjugated ester ($\nu_{max}$ 1710 $cm^{-1}$), and an ether group ($\nu_{max}$ 1130 and 1108 $cm^{-1}$). The $^1H$ NMR spectrum showed four tertiary methyls ($\delta_H$ 1.25, 1.17, 1.13 and 0.82, each 3H), four $sp^2$ methine protons at $\delta_H$ 6.01 (1H, d, $J$ = 5.6 Hz), 5.43 (1H, br.s), 5.73 (1H, d, $J$ = 9.4 Hz) and 7.13 (1H, d, $J$ = 9.4 Hz), one oxygenated methylene proton at $\delta_H$ 3.62 (1H, d, $J$ = 7.2 Hz) and 3.43 (1H, d, $J$ = 7.2 Hz), three oxygenated methine protons at $\delta_H$ 4.20 (1H, d, $J$ = 3.6 Hz), 4.46 (1H, dd, $J$ = 12.3, 3.6 Hz) and a hemiacetal protons at $\delta_H$ 6.01 (1H, d, $J$ = 5.6 Hz). In addition, three $sp^3$ methylenes at $\delta_H$ 2.50 (1H, m), 2.20 (1H, m), 1.80 (1H, m), 1.62 (1H, m), 2.41 (1H, m) and 1.70 (1H, m) as weel as three $sp^3$ methines at $\delta_H$ 3.16 (1H, m), 2.22 (1H, m) and 2.59 (1H, d, $J$ = 12.3 Hz) also were observed in $^1H$ NMR spectrum. The $^{13}C$ NMR together with the DEPT spectra revealed 26 carbon signals, including a carbonyl [$\delta_C$ 203.7 (s)], a carbonyl ester [$\delta_C$ 171.6 (s)], four $sp^2$ methines [$\delta_C$ 153.4 (d), 129.2 (d), 119.5 (d) and 118.7 (d)], two $sp^2$ quaternary carbon [$\delta_C$ 158.8 (s) and 132.7 (s)], three $sp^3$ oxygenated methine carbons [$\delta_C$ 99.4 (d), 73.9 (d) and 72.6 (t)], one oxygenated methylene carbon at $\delta_C$ 79.2 (d), three $sp^3$ methylenes, three $sp^3$ methines, four $sp^3$ quaternary and four methyls carbons. These functionalities accounted for five out of the total eleven degrees of unsaturation. The remaining six degrees of unsaturation were consistent with the limonoid containing six rings (Mohamad et al. 2009). A comparison of the NMR data of **1** with those of ceramicine D isolated from *Chisocheton ceramicus* (Mohamad et al. 2009) revealed that the structures of the two compounds are closely related, the main difference were the absence of an oxygenated

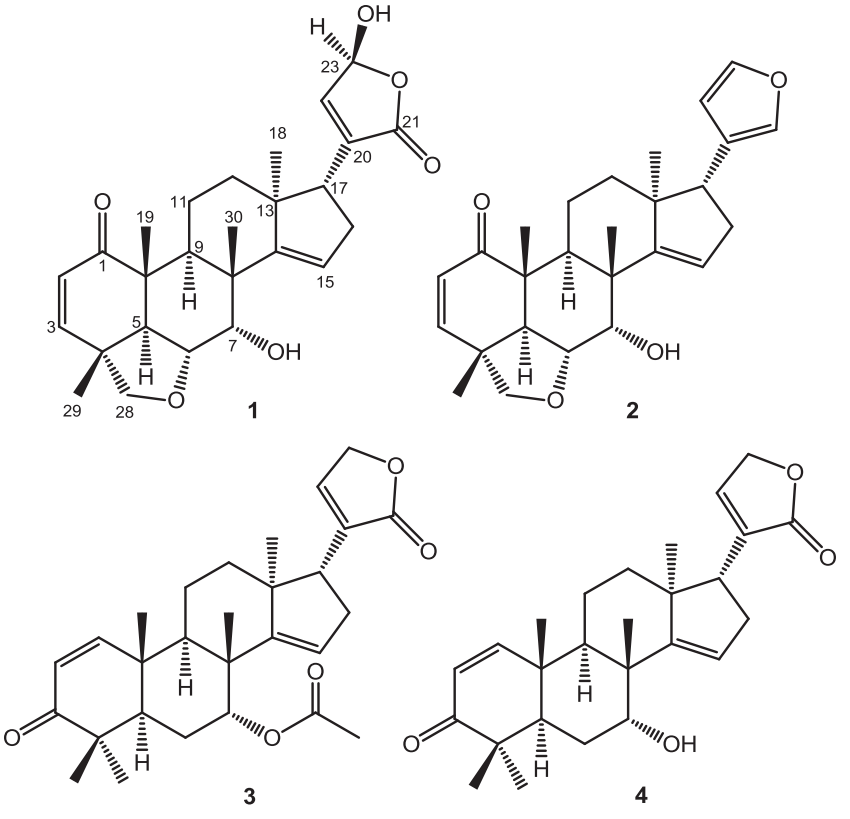


**Figure 1.** Structures of compounds **1**–**4**.

methylene at [$\delta_C$ 70.3, $\delta_H$ 4.77 (br. s)] and appeareance of an oxygenated methine in downfield region at [$\delta_C$ 99.4, $\delta_H$ 6.01 (d, $J$ = 6.8 Hz), suggesting that **1** was a hydroxyl derivative of ceramicine D to form a hemiacetal group at C-23. In order to clarify the position of a newly hydroxyl group, the H–H COSY and HMBC experimets were carried, and the results was shown in Figure S2. The $^1$H–$^1$H COSY spectrum of **1** showed correlations in $H_1$-$H_2$, $H_6$-$H_7$-$H_8$, $H_9$-$H_{10}$-$H_{11}$, $H_{15}$-$H_{16}$-$H_{17}$ and $H_{22}$-$H_{23}$, supporting the presence of limonoid structure in **1**. In the HMBC spectrum, the correlations arising from the tertiary methyl protons to their neighbouring carbons enabled the assignment of the four singlet methyls. Furthermore, an olefinic protons at $\delta_H$ 5.73 and 7.13 are couple each other and were correlated to carbonyl at $\delta_C$ 203.7 (C-1) indicated that an α,β-unsaturated carbonyl was located at C-1, C-2 and C-3, respectively. Correlation from an olefinic proton at H-22 ($\delta_H$ 6.01) to oxygenated carbon C-23 ($\delta_C$ 99.4) and an oxygenated proton at $\delta_H$ 6.01 to C-20 ($\delta_C$ 132.7) and C-21 ($\delta_C$ 171.6) were used to assign a new hydroxyl group was located at C-23. The relative configuration of **1** was determined by NOESY experiment (Figure S3) and by comparison with those similar compound previously reported, ceramicines D (Mohamad et al. 2009) and walsogyne A (Mohamad et al. 2008). NOESY correlations of H-6/$CH_3$-19, H-7/$CH_3$-30 indicated that H-6 and H-7 were each β-configuration. The α-configuration of H-9 and H-5 were assigned by the NOESY cross peaks of H-9/$CH_3$-18 and H-5/H-9. The NOESY correlations between $CH_3$-18/H-23 and $CH_3$-18/H-9, supported that the hydroxyl group at C-23 was β-oriented. The stereochemistry at hemiacetal C-23 was assigened not to be epimerised because only single signal at NMR

spectra (C-20, $\delta_C$ 132.7; C-22, $\delta_C$ 118.7; C-23, $\delta_C$ 99.4) and quite different to those of walsogyne A, isolated from *Walsura chrysogyne* (Mohamad et al. 2008). The NOESY cross peak also observed between $CH_3$-30/H-17 indicated that the α-pyrone ring at C-17 was α-oriented. Other correlations in the NOE spectra supported that the relative configuration of **1** was similar to those of ceramicines D (Mohamad et al. 2009), therefore, the structure of **1** was elucidated as the new limonoid and, namely pentandricine.

The known compounds ceramicines B (**2**) (Mohamad et al. 2009), 6-de(acetyloxy)-23-oxochisocheton (**3**) (Gunning et al. 1994) and 6-de(acetyloxy)-23-oxo-7-*O*-deacetylchisocheton (**4**) (Gunning et al. 1994) were identified by comparison of their spectroscopic data with reported values.

The cytotoxic activity of the isolated compounds **1**–**4** was evaluated against the against MCF-7 breast cancer cells according to a method described (Skehan et al. 1990) and Cisplatin ($IC_{50}$ 27.0 μM) was used as a positive control (Hadisaputri et al. 2012). Based on the $IC_{50}$ value of compounds **1**, **3** and **4**, compound **4** showed stronger activity, suggesting that the presence of an acetyl, hydroxyl in lactone ring and ether ring seems to increase the cytotoxic activity.

## 3. Experimental

### 3.1. General experimental procedures

Optical rotations were recorded on an ATAGO AP-300 automatic polarimeter. UV spectra were measured using a TECAN Infinite M200 pro, with MeOH. The IR spectra were recorded on SHIMADZU IRPrestige-21 in KBr. The mass spectra were recorded with a Waters Xevo QTOF MS. NMR data were recorded on a Bruker Topspin spectrometer at 500 MHz for $^1H$ and 125 MHz for $^{13}C$ using TMS as internal standard. Column chromatography was conducted on silica gel 60. TLC plates were precoated with silica gel $GF_{254}$ (Merck, 0.25 mm) and detection was achieved by spraying with 10% $H_2SO_4$ in EtOH, followed by heating and irridiation under ultraviolet–visible light at a wavelength of 257 and 364 nm.

### 3.2. Plant material

The stem bark of *C. pentandrus* was collected in Bogor Botanical Garden, Bogor, West Java Province, Indonesia in June 2016. The plant was identified by the staff of the Bogoriense Herbarium, Bogor, Indonesia and a voucher specimen (No. Bo-104) was deposited at the Herbarium.

### 3.3. Extraction and isolation

The dried ground stembark (1.8 kg) of *C. pentandrus* was extracted with methanol exhaustively (14 L) at room temperature for 7 days. After removal of the solvent under vacuum, the viscous concentrate of MeOH extract (340.01 g) was first suspended in $H_2O$ and then partitioned with *n*-hexane, EtOAc, and *n*-butanol, successively. Evaporation resulted in the crude extracts of *n*-hexane (10.90 g), EtOAc (25.18 g), and *n*-butanol (228.63 g), respectively. The *n*-hexane soluble fraction (10.90 g) was fractionated by column chromatography on silica gel using a gradient *n*-hexane, EtOAc and MeOH to give fractions A–H, combined according

to TLC results. Fraction B (5.39 g) was subjected to column chromatography over silica gel using a gradient mixture of *n*-hexane-$CH_2Cl_2$-EtOAc (5% stepwise) as eluting solvents to afford thirteen subfractions (B1-B13). Subfraction B9 (912.2 mg) was chromatographed on a column of silica gel, eluted with *n*-hexane:$CH_2Cl_2$:EtOAc (5:4:1), to give 6 subfractions (B9A–B9F), subfraction B9C to give (**2**) (62.2 mg). Subfraction B9E was chromatographed on a column of silica gel, eluted with *n*-hexane:EtOAc (6:4), to give (**3**) (2.2 mg). Fraction C (698.8 mg) was subjected to column chromatography over silica gel using a gradient mixture of *n*-hexane-$CH_2Cl_2$-EtOAc (5% stepwise) as eluting solvents to afford nine subfraction (C1-C9). Subfraction C2 (126.1 mg) was chromatographed on a column of silica gel, eluted with *n*-hexane:$CH_2Cl_2$:EtOAc (5:1.5:3.5), to give six subfractions (C2A–C2F), Subfraction C2B (16.8 mg) was chromatographed on preparative TLC, eluted with *n*-hexane:$CH_2Cl_2$:EtOAc (4:2.5:5), to give (**3**) (3.4 mg) and (**4**) (4.6 mg). Fraction E (819.8 mg) was chromatographed on a column of silica gel, eluted with *n*-hexane:$CH_2Cl_2$:EtOAc (5:1.5:3.5), Subfraction E4 (61.2 mg) was chromatographed on a column of silica gel, eluted with $CH_2Cl_2$:EtOAc (6.5:3.5), to give (**1**) (23.4 mg).

Pentandricine-A (**1**), colourless amorphous solid, $[\alpha]_D^{20} + 39°$ (*c* 0.1, DMSO), UV (MeOH) $\lambda_{max}$ 230 nm (log $\varepsilon$ 4.2); IR (KBr) $\nu_{max}$ 3535, 3457, 2862, 1720, 1457, 1387, 1247 $cm^{-1}$; HR-TOFMS *m/z* 439.2025 $[M + H]^-$, (Calcd $C_{26}H_{32}O_6$ *m/z* 440.2121). $^1H$ NMR (DMSO, 500 MHz): $\delta_H$ 7.13 (1H, d, *J* = 9.4 Hz, H-3), 6.06 (1H, d, *J* = 5.6 Hz, H-22), 6.01 (1H, d, *J* = 5.6 Hz, H-23), 5.73 (1H, d, *J* = 9.4 Hz, H-2), 5.43 (1H, br.s, H-15), 4.46 (1H, dd, *J* = 12.3, 3.6 Hz, H-6), 4.20 (1H, d, *J* = 3.6 Hz, H-7), 3.62 (1H, d, *J* = 7.2 Hz, H-28a), 3.43 (1H, d, *J* = 7.2 Hz, H-28b), 3.16 (1H, m, H-17), 2.59 (1H, d, *J* = 12.3 Hz, H-5), 2.50 (1H, m, H-16a), 2.41 (1H, m, H-11a), 2.22 (1H, m, H-9), 2.20 (1H, m, H-16b), 1.70 (1H, m, H-11b), 1.80 (1H, m, H-12a), 1.62 (1H, m, H-12b), 1.25 (3H, s, $CH_3$-29), 1.17 (3H, s, $CH_3$-19), 1.13 (3H, s, $CH_3$-30), 0.82 (3H, s, $CH_3$-18); $^{13}C$ NMR (DMSO, 125 MHz): 203.7 (C-1), 171.6 (C-21), 158.8 (C-14), 153.4 (C-3), 132.7 (C-20), 129.2 (C-2), 119.5 (C-15), 118.7 (C-22), 99.4 (C-23), 79.2 (C-28), 73.9 (C-6), 72.6 (C-7), 53.4 (C-17), 41.9 (C-4), 47.7 (C-5), 47.1 (C-13), 46.9 (C-8), 46.9 (C-11), 35.8 (C-10), 34.3 (C-12), 33.2 (C-16), 26.1 (C-30), 22.4 (C-18), 18.1 (C-12), 14.3 (C-19), 14.3 (C-29).

### *3.4. Bioassays for cytotoxic activity (Skehan et al. 1990)*

The MCF-7 cells were seeded into 96-well plates at an initial cell density of approximately $3 \times 10^4$ cells $cm^{-3}$. After 24 h of incubation for cell attachment and growth, varying concentrations of samples were added. The compounds added were first dissolved in DMSO at the required concentration. Subsequent six desirable concentrations were prepared using PBS (phosphoric buffer solution, pH 7.30–7.65). Control wells received only DMSO. The assay was terminated after a 48 h incubation period by adding MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; also named as thiazol blue] and the incubation was continued for another 4 h, in which the MTT-stop solution containing SDS (sodium dodecyl sulphate) was added and another 24 h incubation was conducted. Optical density was read using a micro plate reader at 550 nm. $IC_{50}$ values were taken from the plotted graph of percentage live cells compared to control (%), receiving only PBS and DMSO, vs. the tested concentration of compounds (μM). The $IC_{50}$ value is the concentration required for 50% growth inhibition. Each assay and analysis was run in triplicate and averaged.

## 4. Conclusions

A new limonoid compound, namely pentandricine (**1**), along with three known limonoid-compounds, **2**–**4**, were isolated from the stembark of *C. pentandrus*. Compounds **1**–**4** showed weak and no cytotoxic activity against MCF-7 breast cancer cells with $IC_{50}$ values of 369.84, 150.86, 208.93 and 120.09 µM, respectively, indicating the presence of an acetyl group, hydroxyl group in lactone ring and ether ring in compounds **1**, **3** and **4**, seems to increase the cytotoxic activity.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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