Nimonol from *Chisocheton macrophyllus* (Meliaceae) Seeds and Their Cytotoxic Activity against P-388 Leukaemia Cells

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Abstract

The *Chisocheton* genus belongs to the Meliaceae family which produces various structures and activities of compounds, such as antimalarial, antimicrobial, antitumor, anti-inflammatory, and cytotoxic. This plant has 53 species that are spread in tropical and sub-tropical forests, including Indonesia. *Chisocheton* plants have been known as plants that produce limonoids, namely triterpenoid compounds that have been modified to lose four terminal carbons (tetrannortriterpenoids). One of the species whose phytochemical reports are still few and interesting for research on limonoid content is *Chisocheton macrophyllus*. *Chisocheton macrophyllus* is a tall plant that grows in the rainforest in the northern part of the island of Sulawesi, Indonesia, has the regional name ma aa, gula, pasak lingga, gending, ta suea, bekak, or pithraj tree. This paper will describe a limonoid compound, namely nimonol which has been isolated from *Chisocheton macrophyllus*. Nimonol is known to have the molecular formula C\(_{33}\)H\(_{30}\)O\(_3\) from a group of havanensin. The structure was determined by spectroscopic methods UV, IR, \(^1\)D-NMR (\(^1\)H-NMR, \(^13\)C-NMR, and DEPT), 2D-NMR (\(^1\)H-\(^1\)H COSY, HMQC, and HMBC), and mass spectroscopy.

**Keywords:** *C. macrophyllus*, havanensin, limonoid, Meliaceae.

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1. INTRODUCTION

*Chisocheton* plants are among the second largest in the Meliaceae family, a family that has been known as a plant that produces various secondary metabolites and interesting biological activities. This plant has 53 species distributed in tropical and subtropical forests such as Asia, Malaysia, and Indonesia (Shilpi et al., 2016). *Chisocheton* compounds have been widely reported to have a variety of activities, such as antimalarial (Nurlelasari et al., 2016), antimicrobials (Nurlelasari et al., 2021; Fitriania et al., 2021; Nurlelasari et al., 2020; Rahmayanti et al., 2021), antifeedant, growth regulator, repellent, larvacide (Nurlelasari et al., 2017; Lin et al., 2021; Lin et al., 2022), antitumor (Rahmayanti et al., 2021; Cahn et al., 2012; Najmuldeen et al., 2012) and anti-inflammatory (Yang et al., 2012) and cytotoxic activity (Nurlelasari et al., 2016; Badria & Ahmed, 2018; Katja et al., 2016; Nurlelasari et al., 2021).

In a previous study of the *Chisocheton* genus, the highest number of limonoids and triterpenoids were found (Shilpi et al., 2016; Najmuldeen et al., 2012; Supratman et al., 2020; Nurlelasari et al., 2021; Nurlelasari et al., 2015), then steroid, phenolic (Hidayat et al., 2018) and spermidine alkaloids (Nguyen et al., 2020).

*Chisocheton macrophyllus* is an Indonesian tropical forest plant whose secondary metabolite studies are still rare, so there is a great opportunity to obtain its limonoid compounds. *C. macrophyllus* is a higher plant found growing in the rain forest in the northern part of Malaysia and Indonesia (Shilpi et al., 2016; Nurlelasari et al., 2017;
2. MATERIALS AND METHODS

Materials and Tools

Seeds of *C. macrophyllus* were obtained from the Bogor Botanical Gardens, Bogor, Indonesia, in August 2011. This plant was determined by its herbarium without specimen code No. Bo-1295452.

General Experimental Procedures

The equipment used in this study includes glassware commonly used in isolation techniques in the Natural product. In addition, other supporting equipment is used, such as an analytical balance, a Buchi R-200 rotary evaporator with a Buchi V-500 vacuum pump, a Buchi B-490 water bath, and an F-100 cooling circulator. Thin layer chromatography (TLC) and UV Vilber Lourmat detector lamp (λ 254 nm and 365 nm) with 10% (v/v) sulfuric acid in ethanol and Ehrlich's reagent as stain reagent were obtained with a JEOL JMS spectrum spectroscopic infrared (IR) Perkin-Elmer spectrum-100 FT-IR in the KBr plate. Mass spectra were obtained with a JEOL JMS-700 and SynaptG2 mass spectrometer instruments, and NMR spectra were measured with a JEOL JNM ECA-500 spectrometer at 500 MHz for $^1$H-NMR and 125 MHz for $^{13}$C-NMR with TMS as standard.

Extraction and Isolation

Seeds powder of *C. macrophyllus* (3.5 kg) was extracted with MeOH at room temperature to obtain a concentrated extract of 414 g of methanol. Then, the concentrated MeOH extract was partitioned successively using *n*-hexane, EtOAc, and *n*-butanol to obtain 197 g, 108 g and 8 g of concentrated extracts of *n*-hexane, EtOAc and *n*-butanol, respectively. Each extract was guided for the presence of limonoids using the Ehrlich stain reagent, producing a red colour indicating the presence of limonoids. The *n*-hexane extract (60 g) was separated by vacuum liquid chromatography (KCV) method and eluted with *n*-hexane, EtOAc and 10% methanol to obtain seven fractions (A01-A7). The A03-A05 fractions were combined (10.5 g) and were separated using column chromatography with the *n*-hexane/EtOAc solvent system (7:3) to obtain 9 subfractions (B01-B9). Subfractions B04 and B05 were combined (0.2 g) and were separated again using column chromatography with a chloroform-acetone solvent system (9:8:0.2) to give nimonol (16.1 mg).

Cytotoxic Assay Against P-388 Murine Leukaemia Cells

The activity test of isolated compounds was determined by testing their cytotoxic properties against murine leukaemia P-388 cells using the Alley method. P-388 murine leukaemia cells are used in the initial screening for compounds that have the potential as anticancer at the National Cancer Institute (NCI). The working principle of the cytotoxic measurement of P-388 murine leukaemia cells is as follows: the activity of pure compounds and artonin E (0.03 g/mL) as a comparison compound is expressed by the determination of IC$_{50}$, which is the concentration of the sample or comparison required to inhibit 50% of P-388 murine leukaemia cancer cells by staining with MTT reagent [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazoliumbromide; also called bluethiazole], which was observed with a microplate reader at 540 nm.

The test was carried out by adding various concentrations of nimonol and artonin E to murine P-388 cancer cells. After being incubated for 48 hours, MTT colour reagent was added to the sample and incubated for 4 hours. Cells still alive will change the colour of the MTT from yellow to blue. The number of
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Figure 1. Structures of nimonol

Obtained Nimonol (Figure 1) is a colourless crystal soluble in chloroform and acetone. The molecular ion obtained [M+H]⁺ at m/z 453.2596 (calc. for C₂₈H₃₆O₅, 452.5824) in the HR-ESI-TOFMS spectrum, showing the molecular formula of C₂₈H₃₆O₅, with eleven degrees of unsaturation. UV spectrum analysis showed the presence of the α,β-unsaturated ketone system, is at λmax 240 nm. In contrast, analysis based on the IR spectrum shows the presence of functional groups of hydroxyl (OH) (νmax 3414 cm⁻¹), ester carbonyls (νmax 1739 cm⁻¹), conjugated carbonyl (νmax 1658 cm⁻¹) and olefinic (νmax 1450 cm⁻¹)

The existence of the α,β-unsaturated ketone system was confirmed from the ¹H-NMR spectral data, which appeared at the δH 205.2 ppm and two olefinic protons [δH 5.89 (1H; d; 10.4); 7.10 (1H; d; 10.4)]. Furthermore, the suspicion of limonoid compounds was shown based on the ¹H-NMR spectrum data, namely the presence of five tertiary methyl groups (δH 0.83, 1.19, 1.19, 1.19, and 1.30, each 3H, s), as well as the characteristics of the furan moiety at [δH 6.28 (1H, d, J= 1.3 Hz), 7.26 (1H, s), and 7.39 (1H, m)] (Nurlelasari et al., 2017; Rahmayanti et al., 2021). There are acetoxyl groups (δH 2.18; 3H, s), two oxygenated proton (δH 4.07 and 5.46), and one olefinic protons at [5.57 (1H, d, J=1.9 Hz)].

The ¹H-NMR spectrum (Table 1) showed the presence of five tertiary methyl groups (δH 0.83, 1.19, 1.19, 1.19, and 1.30, each 3H, s), one acetoxyl group (δH 2.18; 3H, s), two oxygenated protons (δH 4.07 and 5.46), β-furan moiety at [δH 6.28 (1H, d, J= 1.3 Hz), 7.26 (1H, s), and 7.39 (1H, m)], and two olefinic protons at [δH 5.89 (1H, d, J=10.4 Hz) and 5.57 (1H, d, J=1.9 Hz)].

Table 1. NMR data (500 MHz for ¹H and 125 MHz for ¹³C, in CDCl₃) for nimonol

| Position | Nimonol |
|----------|---------|
| H/C | ¹H (mult, J Hz) | ¹C (mult, J Hz) |
| 1 | 7.10 (1H, d, 10.4) | 157.4 (d) |
| 2 | 5.89 (1H, d, 10.4) | 126.4 (d) |
| 3 | - | 205.2 (q) |
| 4 | - | 41.3 (q) |
| 5 | 2.24 (1H, d, 12.0) | 48.5 (d) |
| 6 | 4.07 (1H, m) | 68.7 (d) |
| 7 | 5.46 (1H, d, 2.34) | 78.7 (d) |
| 8 | - | 45.1 (q) |
| 9 | 2.29 (1H, m) | 35.8 (d) |
| 10 | - | 42.3 (q) |
| 11 | 1.73 (1H, m) | 16.4 (t) |
| 12 | 1.93 (1H, m) | 32.4 (t) |
| 13 | - | 47.4 (q) |
| 14 | - | 159.3 (q) |
| 15 | 5.57 (1H, d, 1.9) | 120.8 (d) |
| 16 | 2.43, 1.67 (1H, m) | 34.6 (d) |
| 17 | 2.86 (1H, dd, 18.5) | 51.8 (d) |
| 18 | 0.83 (3H, s) | 20.8 (s) |
| 19 | 1.30 (3H, s) | 27.9 (s) |
| 20 | - | 124.4 (q) |
| 21 | 7.26 (1H, s) | 139.9 (q) |
| 22 | 6.28 (1H, d, 1.3) | 111.2 (q) |
| 23 | 7.39 (1H, m) | 142.9 (q) |
| 24 | 1.19 (3H, s) | 20.8 (s) |
| 25 | 1.19 (3H, s) | 31.9 (s) |
| 30 | 1.19 (3H, s) | 20.8 (s) |
| 1' | 2.18 (3H, s) | 21.9 (s) |
| 2' | - | 171.7 (q) |
Based on the $^{13}$C-NMR data detailed by DEPT and HMQC, it can be seen that there are twenty-eight carbon resonances (can be seen in Table 1) consisting of one carbonyl ($\delta_C$ 205.2), one acetyl carbon ($\delta_C$ 21.9 and 171.7), six sp$^2$ methines ($\delta_C$ 111.2, 124.4, 126.4, 139.9, 142.9, and 157.4), five methyls, two sp$^3$ methylenes, two sp$^3$ oxygenated carbons ($\delta_C$ 68.7 and 78.7), five sp$^3$ methines, four sp$^3$ quarternary carbons, and two sp$^3$ quarternary carbons ($\delta_C$ 124.4 and 159.3). From these data, it can be seen that the functionality is six out of eleven degrees of unsaturation, so there are five degrees of unsaturation remaining which correspond to the presence of pentacyclic limonoids (Nurlelasari et al., 2017).

The $^1$H-NMR and $^{13}$C-NMR data for the isolated compound was based on the characteristics of limonoid compounds, which had a furan group and five tertiary methyl groups as the main alkyl groups in limonoids. The difference with the azadiradiron compound is the presence of an OH group at C-6 of this compound. There is also no acetyl signal at C-6 (C 21.9 and 78.7) for the dysobinin compound (Nurlelasari et al., 2017), indicating that the isolated compound is nimonol.

To determine the connectivity of the partial structure due to hydroxyl groups, $^1$H-$^1$H COSY and HMBC experiments were carried out, and the results are shown in Figure 2. In the HMBC spectrum, the hydroxyl signal ($\delta_H$ 4.07) showed $^2$J correlations with C-7 ($\delta_C$ 78.7) and methines carbon C-9 ($\delta_C$ 35.8). Methyl proton ($\delta_H$ 2.18) showed $^2$J correlations with C-2' ($\delta_C$ 171.7), and methines proton ($\delta_H$ 5.46) showed $^2$J correlations with C-2' ($\delta_C$ 171.7), indicating that the acetyl group was located at C-7. Methine proton ($\delta_H$ 7.10) showed $^2$J correlations with C-9 ($\delta_C$ 35.8), quarternary carbon C-3 ($\delta_C$ 205.2), and quarternary carbon C-4 ($\delta_C$ 41.3), indicating that olefinic was located at CA$^{1,2}$. Methine proton ($\delta_H$ 5.57) showed $^3$J correlations with C-17 ($\delta_C$ 51.8), and $^2$J correlations with C-14 ($\delta_C$ 159.3) and methylene carbon C-16 ($\delta_C$ 34.6) indicated that olefinic was located at CA$^{14,15}$.

Therefore, the compound was established as a limonoid named nimonol. The stereochemistry of nimonol was determined by the biogenesis of the compound containing the oxygenated proton position at C-7 (Iruńgu, 2014), and all conformed to the proposed structure. Thus, the structure of the limonoid compound was identified as nimonol (Nurlelasari et al., 2017; Laphookhieo et al., 2008) based on MS and NMR spectral data and compared the spectrum data with compounds that had been isolated previously.

**Figure 2.** Selected HMBC and $^1$H-$^1$H COSY correlations

The isolated compound's activity test was determined by testing their cytotoxic properties against murine leukaemia P-388 cells using the Alley method and obtained an IC$_{50}$ value of 64.5 g/mL. This value indicates that nimonol activity is not active against P-388 leukaemia cells.

**4. CONCLUSIONS**

A limonoid compound, Nimonol, has been found in the seeds of Chisocheton macrophyllus (Meliaceae). The chemical structure of nimonol was determined by using spectroscopic data and comparing its structure with spectral data from previous studies. This nimonol compound was first discovered in C. macrophyllus seeds, but its activity against murine leukaemia P-388 cells was classified as inactive.

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