**Dammarane-Type Triterpenoids from the Stembark of *Aglaia argentea* (Meliaceae)**

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**Abstract**

Two dammarane-type triterpenoids, 20*S*,24*S*-epoxy-3α,25-dihydroxydammarane (**1**) and 3α-acetyl-20*S*,24*S*- epoxy-3α,25-dihydroxydammarane (**2**), have been isolated from the stembark of *Aglaia argentea*. The chemical structure of compounds **1** and **2** were identified by spectroscopic evidences including, UV, IR, 1D-NMR, 2D-NMR and MS as well as by comparing with previously reported spectral data. Those compounds were isolated from this plant for first time. Compounds **1** and **2** showed cytotoxic activity against P-388 murine leukemia cells with IC50 values of 23.96 and 8.14 M, respectively.

**Keywords**: *Aglaia argentea*, *Aglaia*, dammarane-type triterpenoids, Meliaceae, P-388 Murine leukemia cells.

1. **INTRODUCTION**

 Dammarane-type triterpenoids widely distributed in various medicinal plants and have a great amount of interest in the field of new drug research and development (Zhao et al., 2007). Dammarane-type triterpenoids belong to tetracyclic ring triterpenoids and their structural characteristic is with H-5α, CH3-8, H-9α, CH3-10β, H-13β, CH3-14α, C-17β side chain, and 20*R* or *S* configuration and usually, C-3, C-6, C-7, C-12, C-20, C-23, C-24, or C-25 are replaced by hydroxyl group; C-3, C-6, or C-20 are substituted by saccharide groups and olefinic bond are formatted between C-5 and C-6, C-20 and C-21, C-20 and C-22, C-22 andC C-23, C-24 and C-25 or C-25 and C-26 (Liu et al., 2011). Moreover, cyclization generally displays at C17-side chain. Specifically, a five-membered ring with epoxy bond is usually formed between C-20 and C-24, a five-membered lactone ring usually appears between C-21 and C-23, and a six-membered ring with epoxy bond displays between C-20 and C-25 for dammarane-type triterpenoids (Phan et al., 2011). They are usually classified into protopanaxdiol and protopanaxtriol (with 6-OH) groups based on their aglycone moieties. Furthermore, in pharmacological research, dammarane-type triterpenoids, as well as their derivatives, showed various bioactivities such as anti-tumor, anti-inflammatory, immunostimulatory, neuronal cell proliferatory, anti-aging, anti-bacterial, anti-diabetes, and anti-osteoporosis abilities (Chen et al., 2005; Jin et al., 2011).

The genus *Aglaia* is the largest genus of the family of Meliaceae comprises more than 100 species distributed mainly in India, Indonesia, Malaysia and parts of the Western Pacific region (Muellner et al., 2005). Some species of *Aglaia* have been phytochemically investigated previously with major constituents of dammarane-type triterpenoids (Roux et al., 1998; Zhang et al., 2010; Harneti et al., 2012) and cycloartane-type triterpenoids (Khalit et al., 1999; Weber et al., 2000; Awang et al., 2012) and glabretal-type triterpenoids (Su et al., 2006). In our continous search for novel secondary metabolites from Indonesian *Aglaia* plants, we isolated and described triterpenoids, aglinone and aglinin E, from the bark of *A. smithii* (Harneti et al., 2012), and protolimonoid from the stembark of *A. argentea* (Farabi et al., 2017). In the further screening for novel triterpenoid compounds from Indonesia *Aglaia* plants, we found that the *n*-hexane of *A. argentea* exhibited the presence of triterpenoids. We report herein the isolation, structural elucidation of dammarane-type triterpenoid compounds **1**-**2** (Figure 1).

1. **MATERIAL AND METHODS**

 **General Experimental Prosedure**. Melting points were measured on an electrothermal melting point apparatus and are uncorrected. The IR spectra were recorded on a Perkin-Elmer spectrum-100 FT-IR in KBr. Mass spectra were obtained with a Synapt G2 mass spectrometer instrument. NMR data were recorded on a JEOL ECZ-600 spectrometer at 600 MHz for 1H and 150 MHz for 13C and JEOL JNM A-500 spectrometer at 500 MHz for 1H and 150 MHz for 13C, chemical shifts are given on a  (ppm) scale and tetramethylsilane (TMS) as an internal standard. Column chromatography was conducted on silica gel 60 (Kanto Chemical Co., Inc., Japan). TLC plates were precoated with silica gel GF254 (Merck, 0.25 mm) and detection was achieved by spraying with 80% H2SO4 in water, followed by heating.

 **Plant Material**. The stembark of *A. argentea* were collected in Bogor Botanical Garden, Bogor, West Java Province, Indonesia in June 2015. The plant was identified by the staff of the Bogoriense Herbarium, Bogor, Indonesia and a voucher specimen (No. Bo-1288718) was deposited at the Herbarium.

 **Extraction and isolation**. The dried and powdered of *A. argentea* (2.5 kg) was extracted with methanol (14 L) at room temperature for 5 days. After removing the solvent, the methanol extract (133.5 g) was recovered. The extract was then suspended water (500 mL) and successively extracted with *n*-hexane (2 × 1 L), ethyl acetate (2 × 1 L) and *n*-butanol (2 × 1 L) to afford *n*-hexane (27 g), ethyl acetate (16 g) and *n*-BuOH (36 g) extracts, respectively. The *n*-hexane soluble fraction (26.3 g) was separated by vacum liquid chromatography on silica gel 60 using a gradient *n*-hexane and EtOAc to give nine fractions (A–I). Fraction B (2.50 g) was chromatographed on a column of silica gel, eluted with a gradient of *n*-hexane–EtOAc (10:0–1:1), to give six subfractions (C01–C06). Subfraction C03 (250 mg) was chromatographed on a column of silica gel, eluted with CH2Cl2:CHCl3 (9.5:0.50), to give five subfractions (C03A-C03D). Subfraction C03C was separated on preparative TLC on silica gel GF254, eluted with *n*-hexane–EtOAc (8.5:1.5), to give **1**  (15.2 mg). Fraction C and D were combined (1.80 g) and was chromatographed on a column of silica gel, eluted with a gradient of *n*-hexane–EtOAc (10:1–1:10), to give seven subfractions (D01–D07). Subfraction D05 (340 mg) was chromatographed on a column of silica gel, eluted with a gradient of *n*-hexane–EtOAc (10:1–1:10) to afford four subfraction (D05A-D05D). Subfraction D05C was chromatographed on a column of silica gel, eluted with a gradient of CHCl3–EtOAc (10:1–1:10) to give **2** (10.5 mg).

1. **RESULTS AND DISCUSSION**

 The methanolic extract from the dried stem bark of *A. argentea* was concentrated and extracted successively with *n*-hexane, ethyl acetate, and *n*-butanol. The *n*-hexane exhibited the presence of triterpenoid compounds. By using triterpenoid test to guide separations, the *n*-hexane fraction was separated by combination of column chromatography on silica gel and preparative TLC on silica gel GF254 to afford two dammarane-type triterpenoids, **1** and **2** (Figure 1).

**20*S*,24*S*-epoxy-3α,25-dihydroxydammarane** (**1**): White crystal, melting points, 166-167 °C; IR (KBr) *v*maks 2866, 3457, 1457, 1380, 1055 cm-1; 1H-NMR (CDCl3, 600 MHz), 13C-NMR (CDCl3, 150 MHz), See Table 1; ESI-MS *m/z* 461.36 [M+H]+, (calcd. for C30H52O3 *m/z* 460.39).

**3α-acetyl-20*S*,24*S*- epoxy-3α,25-dihydroxydammarane** (**2**): Solid amorphous powder; IR (KBr) *v*maks 3200, 2949, 1705, 1457, 1380, 1080 cm-1; 1H-NMR (CDCl3, 500 MHz); 13C-NMR (CDCl3, 125 MHz), see Table 1; HR-TOFMS *m/z* 501.3770 [M-H]-, (calcd. for C32H54O4 *m/z* 502,4022).



Figure 1. Chemical Structures of Compounds **1** and **2**

Compound **1** was isolated as a white needle crystal, melting points, 166-167 oC. The molecular formula of **1** was established to be C30H52O3 based on of ESI-MS spectra (*m/z* 461.36 [M+H]+, calcd. for C30H52O3 *m/z* 460.39) along with NMR data (Tabel 1), thus requiring five degrees of unsaturation. The UV spectrum showed no conjugated double based on the absorption maximum above 200 nm. IR spectrum of **1** showed the presence of a hydroxyl group (3457 cm-1), an aliphatic bands (2866 cm-1), a *gem*-dimethyl (1457 and 1380 cm-1) and an ether group (1055 cm-1).

1H-NMR spectrum showed the presence of eight tertiary methyl signals at H 0.82 (3H, s, CH3-28), 0.84 (3H, s, CH3-18), 0.87 (3H, s, CH3-19), 0.92 (3H, s, CH3-29), 0.95 (3H, s, CH3-30), 1.09 (3H, s, CH3-26), 1.13 (3H, s, CH3-21) and 1.17 (3H, s, CH3-27), which characteristic for dammarane-type triterpenoid (Harneti et al., 2014). An oxygenated sp3 methine at H 3.38 (1H, t, *J*=3.0 Hz) and an oxygenated sp3 methine in part of tetrahydrofuran ring at H 3.62 (1H, dd, *J*=4.8, 10.2 Hz) were also obsereved in the 1H-NMR spectra, supporting the presence of dammarane-type triterpenoid structure in compound **1** (Roux et al., 1998).

 13C-NMR spectrum of **1** showed thirty carbon resonances which were classified by their chemical shifts and the DEPT spectrum as eight methyls, ten methylenes, six methines and six quarternary carbons, indicating the presence of dammarane-type triterpenoid (Harneti et al., 2014). The presence of eight methyl resonances at C 15.6 (CH3-30), 16.2 (CH3-18), 16.6 (CH3-19), 22.2 (CH3-28), 24.1 (CH3-26), 27.3 (CH3-21), 27.9 (CH3-27) and 28.4 (CH3-29), as well as two oxygenated quartenary carbon at C 86.7 and 70.3, supporting the presence of dammarane-type triterpenoid with addition of tetrahydrofuran ring (Roux et al., 1998).

In order to clarify the position of functional groups in compound **1**, 1H-1H COSY and HMBC experiments were carried out and the results was shown in Figure 2. The 1H-1H COSY spectrum of **1** displayed the correlations in C1-C2-C3, C5-C6-C7, C9-C11-C12-C13, C14-C15-C16-C17 and C22-C23-C24, supporting the presence of dammaran-type triterpenoid structure in **1**. In the HMBC spectrum, the correlations arising from the tertiary methyl protons to their neighboring carbons enabled the assignment of the eight singlet methyls at C-4 (2×), C-8, C-10, C-14, C-20, C-26 and C-27, respectively. A methylene protons at H 1.55 and methyl protons at H 0.82 (CH3-29) were correlated to oxygenated carbon at C 76.4 (C-3), indicated that a secondary hydroxyl group was attached at C-3. Methyl protons at H 1.17 and 1.09, as well as an oxygenated methine at H 3.62 were correlated to oxygenated carbon at C 70.3 (C-25), indicated that a tertiary alcohol and an isopropyl group were attached at C-25 and C-24, respectively. A methine proton at H 1.44 was correlated to C-20 (C 86.7), whereas the methyl proton at H 1.13 was correlated to C-20 (C 86.7), C-17 (C 49.8) and C-22 (C 35.3), indicated that a tetrahydrofuran ring was attached at C-17. The presence of a tetrahydrofuran ring at C-17 was supported also by correlation between a methylene proton at H 1.85 and C-24 (C 86.3).

Figure 2. Selected 1H-1H COSY and HMBC correlations for Compounds **1** and **2**

Relative stereochemistry of compound **1** was determined on the basis of coupling constant (3*J*) and chemical shift in the 1H and 13C-NMR spectra. A methine proton at C-3 has a 3*J* 3.0 Hz, indicating that H-2 and H-3 has axial-equatorial orientation, consequently 3-OH has -orientation (Zhang et al., 2010). A detail anaylisis of NMR spectra with side chain of 20,34-epoxy-25-hydroxy, indicated that C values can be used for determining of 24*R* and 24*S* isomer, where C  83.2 for *R* isomer and 86.5 for *S* isomer. In addition, the chemical shift and coupling constant of H-2 can be used also for determining 24*R* and 24*S* isomer with chemical shift, H 3.7 (1H, t, *J*=7.0 Hz) and H 3.6 (1H, dd, *J*=5.5, 10.0 Hz), respectively (Roux et al., 1998). Compound **1** showed the chemical shift for C-23 and H-24 [C 86.3 and H 3.62 (1H, dd, *J*=4.8, 10.2 Hz), as well as C 86.7 for C-20, consequently configuration for C-20 and C-24 are *S* orientation.

A comparison of the NMR data of **1** with those of 20*S*,24*R*-epoxy-25-hydroxydammarane isolated from *Aglaia foveolata* (Roux et al., 1998) revealed that the structures of the two compounds are closely related, the main differences is the chemical shift of C-24 (C 83.3), whereas compound **1** was C 86.3, consequently compound **1** was identified as 20*S*,24*S*-epoxy-25-hydroxydammarane, which showed from this plant for the first time.

Table 1. NMR data for Compounds **1** and **2**

|  |  |  |
| --- | --- | --- |
| Position of Carbon | **1\*** | **2\*\*** |
| 13C NMRC (mult.) | 1H NMRH (Integ. Mult., *J*=Hz) | 13C NMRC (mult.) | 1H NMRH (Integ. Mult., *J*=Hz) |
| 1 | 33.7 (t) | 1.42 (1H, m) | 34.3 (t) | 1.40 (1H, m) |
|  |  | 1.54 (1H, m) |  | 1.56 (1H, m) |
| 2 | 25.4 (t) | 1.55 (1H, m) | 24.8 (t) | 1.56 (1H, m) |
|  |  | 1.62 (1H, m) |  | 1.61 (1H, m) |
| 3 | 76.4 (d) | 3.38 (1H, t, 3.0) | 78.5 (d) | 4.61 (1H, t, 3.0) |
| 4 | 37.3 (s) | - | 36.8 (s) | - |
| 5 | 49.6 (d) | 1.24 (1H, m) | 50.6 (d) | 1.42 (1H, dd, 3.0, 12.0) |
| 6 | 18.3 (t) | 1.39 (1H, m) | 18.2 (t) | 1.38 (1H, m) |
|  |  | 1.54 (1H, m) |  | 1.53 (1H, m) |
| 7 | 34.8 (t) | 1.63 (1H, m) | 35.3 (t) | 1.65 (1H, m) |
|  |  | 1.74 (1H, m) |  | 1.76 (1H, m) |
| 8 | 40.7 (s) | - | 40.6 (s) | - |
| 9 | 50.7 (d) | 1.44 (1H, dd, 2.4, 5.2) | 50.8 (d) | 1.20 (1H, dd, 2.6, 5.8) |
| 10 | 37.7 (s) | - | 37.2 (s) |  |
| 11 | 21.7 (t) | 1.53 (1H, m) | 21.7 (t) | 1.52 (1H, m) |
|  |  | 1.24 (1H, dd, 5.2, 7.0) |  | 1.27 (1H, dd, 4.6, 6.5) |
| 12 | 27.1 (t) | 1.75 (1H, m) | 27.1 (t) | 1.78 (1H, m) |
|  |  | 1.82 (1H, dd, 4.4, 7.0) |  | 1.84 (1H, dd, 6.5, 7.2)  |
| 13 | 42.8 (d) | 1.62 (1H, dd, 4.4, 6.2) | 42.8 (d) | 1.65 (1H, dd, 7.2, 10.8) |
| 14 | 50.2 (s) | - | 50.2 (s) | - |
| 15 | 31.5 (t) | 1.04 (1H, m) | 31.6 (t) | 1.05 (1H, dd, 6.2, 9.8) |
|  |  | 1.24 (1H, m) |  | 1.48 (1H, m) |
| 16 | 25.9 (t) | 1.51 (1H, m) | 25.9 (t) | 1.87 (1H, m) |
|  |  | 1.56 (1H, m) |  | 1.92 (1H, m) |
| 17 | 49.8 (d) | 1.44 (1H, dd, 2.5, 6.2) | 49.9 (d) | 1.46 (1H, dd, 2.7, 6.8) |
| 18 | 16.2 (q) | 0.84 (3H, s) | 15.6 (q) | 0.96 (3H, s) |
| 19 | 16.6 (q) | 0.87 (3H, s) | 16.1 (q) | 0.85 (3H, s) |
| 20 | 86.7 (s) | - | 86.7 (s) | - |
| 21 | 27.3 (q) | 1.13 (3H, s) | 27.4 (q) | 1.14 (3H, s) |
| 22 | 35.3 (t) | 1.22 (1H, m) | 35.2 (t) | 1.22 (1H, m) |
|  |  | 1.34 (1H, m) |  | 1.35 (1H, m) |
| 23 | 26.4 (t) | 1.85 (1H, m) | 26.4 (t) | 1.87 (1H, m) |
|  |  | 1.76 (1H, m) |  | 1.74 (1H, m) |
| 24 | 86.3 (d) | 3.62 (1H, dd, 4.8, 10.2) | 86.4 (d) | 3.63 (1H, 4.8, 10.2) |
| 25 | 70.3 (s) | - | 70.4 (s) | - |
| 26 | 27.9 (q) | 1.17 (3H, s) | 28.0 (q) | 1.18 (3H, s) |
| 27 | 24.1 (q) | 1.09 (3H, s) | 24.1 (q) | 1.10 (3H, s) |
| 28 | 28.4 (q) | 0.92 (3H, s) | 27.9 (q) | 0.82 (3H, s) |
| 29 | 22.2 (q) | 0.82 (3H, s) | 21.8 (q) | 0.86 (3H, s) |
| 30 | 15.6 (q) | 0.95 (3H, s) | 16.7 (q) | 0.90 (3H, s) |
| 1′ |  |  | 171.1 (s) | - |
|  |  |  | 21.5 (q) | * 1. 3H, s)
 |

\*(600 MHz for 1H and 150 MHz for 13C in CDCl3)

\*\* (500 MHz for 1H and 125 MHz for 13C in CDCl3)

Compound **2** was isolated as a solid amorphous powder . The molecular formula of **2** was established to be C32H54O4 based on of ESI-MS spectra (*m/z* *m/z* 502.4022 [M+H]+, calcd. for C30H52O3 *m/z* 502.4022) together with NMR data (Tabel 1), thus requiring six degrees of unsaturation. The UV spectrum showed no conjugated double based on the absorption maximum above 200 nm. IR spectrum of **2** showed the presence of a hydroxyl group (3200 cm-1), an aliphatic bands (2949 cm-1), a *gem*-dimethyl (1457 and 1380 cm-1) and an ether group (1080 cm-1).

A NMR spectra of **2** was very similar to those of **1**, the main differences are the absence one of the hydroxyl group and the presence of an acetyl group at [C 171.1 (s), 21.5 (q) and H 2.08 (3H, s)]. In order to determine the position of newly acetyl group, HMBC experiment was carried, as the results was shown in Figure 2. In the HMBC spectrum, a methyl proton at H 2.08 was correlated to carbonyl ester at C 171.1, whereas the oxygenated methine at H 4.61 was correlated also to carbonyl ester at C 171.1, indicating that an acetyl group was attched at C-3.

A detailed comparison of the NMR spectra of **2** to those of 3α-asetil-20*S*,24*S*-epoxy-25-hydroxydammarane isolated from *Aglaia foveolata* (Roux et al., 1998) revealed that the structures of the two compounds are very similar, consequently compound **2** was identified as 3α-asetil-20*S*,24*S*-epoxy-25-hydroxydammarane, which showed from this plant for the first time.

The cytotoxicity effects of the two isolated compounds **1** and 2 against the P-388 murine leukemia cells were conducted according to the method described in previous paper (Harneti et al., 2012; Farabi et al., 2017) and were used an Artonin E (IC50 0.75 µg/mL) as a positive control (Hakim et al., 2007). Cytotoxic activity of two dammarane-type triterpenoids, 3α-asetil-20*S*,24*S*-epoxy-25-hydroxydammarane (**2**) showed stronger activity than 20*S*,24*S*-epoxy-25-hydroxydammarane (**1**), indicated that the presence of an acetyl group increase the cytotoxic activity in dammarane-type triterpenoid sctructure.

1. **CONCLUSIONS**

Two dammarane-type triterpenoid, 20*S*,24*S*-epoxy-25-hydroxydammarane (**1**) and 3α-asetil-20*S*,24*S*-epoxy-25-hydroxydammarane (**2**) have been isolated from the stembark of *Chisocheton pentandrus*. This results supported the presence of dammarane-type triterpenoid in *Aglaia* genus. Compound **2** showed stronger cytotoxic activity against P-388 murine leukemia cells, indicated that the presence of an acetyl group in dammarane-type triterpenoid structure can increase cytotoxic activity.

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