New bisamide compounds from the bark of Aglaia eximia (Meliaceae)

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\textbf{A B S T R A C T}

Two new bisamide compounds, eximiamide A (1) and eximiamide B (2) were isolated from the bark of Aglaia eximia (Meliaceae). The chemical structures of the new compound were elucidated on the basis of spectroscopic data. All of the compounds were evaluated for their cytotoxic effects against P-388 murine leukemia cells. Compounds 1 and 2 exhibited cytotoxic activity against P-388 murine leukemia cells with IC\textsubscript{50} values of 7.6 and 8.5 μg/mL, respectively.

\section{1. Introduction}

The genus Aglaia (Meliaceae) comprises more than 100 species and mainly distributed in tropical and subtropical regions (Pannell, 1992; Inada et al., 2001). Previous phytochemical studies of Aglaia genus have revealed the presence of a variety of compounds with interesting biological activities including several rocaglate derivatives (Wu et al., 1997; Kim et al., 2005; Su et al., 2006 Chaidir et al., 1999), bisamides (Saifah et al., 1999; Duong et al., 2007), triterpenoids (Harneti et al., 2012 Xie et al., 2007), steroids (Awang et al., 2012; Harneti et al., 2014), limonoids (Fuzzati et al., 1996), sesquiterpenes (Joycharat et al., 2010), lignans (Wang et al., 2002, 2004) and flavonoids (Nugroho et al., 1999).

Recently, several bisamide derived from putrescine, has been found from this genus (Chin et al., 2010). Among these bisamides, a group of compounds found in several Aglaia species have been reported as exhibiting cytotoxic activity (Kim et al., 2006). As part of our continuing search for anticancer candidate compounds from Aglaia eximia, we isolated and described a new stigmastane steroid, 3,4-epoxy-(22R,25)-tetrahydrofuran-stigmast-5-en from the bark of A. eximia (Harneti et al., 2014). In the further screening for cytotoxic compounds from polar fraction of A. eximia, we found that the methanol extract of the bark of A. eximia exhibited a cytotoxic activity against P-388 murine leukemia cells with an IC\textsubscript{50} of 40 μg/mL. We report herein the isolation and structure elucidation of two new bisamide compounds, eximiamide A (1) and eximiamide B (2), together with their cytotoxic activity against P-388 murine leukemia cells.

\section{2. Results and discussion}

Bark of A. eximia were grounded and successively extracted with n-hexane, ethyl acetate and methanol at room temperature. The methanol extract was chromatographed over a vacuum-liquid chromatographed (VLC) column packed with silica gel 60 by gradient elution. The VLC fractions were repeatedly subjected to normal and reverse-phase column chromatography and preparative TLC on silica gel GF\textsubscript{254} to afford two cytotoxic compounds 1–2 (Fig. 1).

Compound 1 was obtained as yellow oil, [\(\alpha\)]\textsubscript{D}\textsuperscript{20} = -10.5 (c, 0.1, MeOH), the molecular formula of 1 was established to be C\textsubscript{27}H\textsubscript{35}N\textsubscript{6}O\textsubscript{11} from HR-TOFMS spectrum which showed a [M – H]\textsuperscript{+} pseudo molecular ion peak m/z 637.7206 (calcd. for C\textsubscript{27}H\textsubscript{35}N\textsubscript{6}O\textsubscript{11} m/z 636.7354), together with NMR data (Table 1), thus requiring five degree of unsaturation. The UV spectrum showed absorption peak at \(\lambda\text{max} \text{nm} \log e\): 262 (5.25), suggesting the presence of an \(\alpha\),\(\beta\)-unsaturated ketone group. The IR spectrum showed absorption peaks due to hydroxyl (3400 cm\textsuperscript{-1}), NH asymmetric (2935 cm\textsuperscript{-1}), amide carbonyl (1681 cm\textsuperscript{-1}), NH bending
Table 1
NMR data (500 MHz for $^1$H and 125 MHz for $^{13}$C in CD$_3$OD) for 1 and 2.

<table>
<thead>
<tr>
<th>Position</th>
<th>$^{13}$C NMR $\delta_c$ (mult.)</th>
<th>$^1$H NMR $\delta_h$ (integral, multiplicity, Hz)</th>
<th>$^{13}$C NMR $\delta_c$ (mult.)</th>
<th>$^1$H NMR $\delta_h$ (integral, multiplicity, Hz)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>75.9 (d)</td>
<td>4.14 (1H, t, 4.5)</td>
<td>72.5 (d)</td>
<td>4.14 (1H, t, 4.5)</td>
</tr>
<tr>
<td>2</td>
<td>64.5 (t)</td>
<td>3.49 (1H, d, 6.5)</td>
<td>63.0 (t)</td>
<td>3.50 (1H, d, 4.8)</td>
</tr>
<tr>
<td>3</td>
<td>38.7 (s)</td>
<td>-</td>
<td>37.5 (s)</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>30.8 (q)</td>
<td>2.16 (3H, s)</td>
<td>29.5 (q)</td>
<td>1.86 (3H, s)</td>
</tr>
<tr>
<td>5</td>
<td>30.8 (q)</td>
<td>2.16 (3H, s)</td>
<td>29.5 (q)</td>
<td>1.86 (3H, s)</td>
</tr>
<tr>
<td>6</td>
<td>30.8 (q)</td>
<td>2.16 (3H, s)</td>
<td>29.5 (q)</td>
<td>1.86 (3H, s)</td>
</tr>
<tr>
<td>1''</td>
<td>64.5 (t)</td>
<td>3.52 (1H, d, 5.9)</td>
<td>63.0 (t)</td>
<td>3.50 (1H, d, 4.8)</td>
</tr>
<tr>
<td>2''</td>
<td>73.9 (d)</td>
<td>3.65 (1H, q, 5.2, 11.0)</td>
<td>74.4 (d)</td>
<td>3.62 (1H, q, 5.8, 10.5)</td>
</tr>
<tr>
<td>3''</td>
<td>64.5 (t)</td>
<td>3.52 (1H, d, 5.9)</td>
<td>63.0 (t)</td>
<td>3.46 (1H, d, 6.0)</td>
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<tr>
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<td>63.0 (t)</td>
<td>3.56 (1H, d, 4.7)</td>
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<tr>
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<td>74.4 (d)</td>
<td>3.62 (1H, q, 5.8, 10.5)</td>
</tr>
<tr>
<td>3''''</td>
<td>64.5 (t)</td>
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<td>63.0 (t)</td>
<td>3.46 (1H, d, 6.0)</td>
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<tr>
<td>1'''''</td>
<td>166.3 (s)</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>2'''''</td>
<td>102.7 (d)</td>
<td>5.69 (1H, d, 8.4)</td>
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<td>5.66 (1H, d, 8.0)</td>
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<td>3'''''</td>
<td>142.8 (d)</td>
<td>8.01 (1H, d, 8.4)</td>
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<td>4'''''</td>
<td>152.0 (s)</td>
<td>-</td>
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<td>-</td>
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<td>4.11 (1H, t, 4.5)</td>
</tr>
<tr>
<td>3''''''</td>
<td>71.4 (d)</td>
<td>4.18 (1H, t, 4.5)</td>
<td>69.9 (d)</td>
<td>4.17 (1H, t, 4.5)</td>
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<tr>
<td>4''''''</td>
<td>86.4 (d)</td>
<td>4.01 (1H, t, 4.5)</td>
<td>84.9 (d)</td>
<td>3.98 (1H, t, 4.5)</td>
</tr>
<tr>
<td>5''''''</td>
<td>62.4 (t)</td>
<td>3.83 (1H, dd 2.6, 9.8)</td>
<td>60.9 (t)</td>
<td>3.80 (1H, dd 2.8, 9.4)</td>
</tr>
<tr>
<td>5''''''</td>
<td>3.73 (1H, dd 3.3, 9.1)</td>
<td>3.45 (4H, s, NH)</td>
<td>3.70 (1H, dd 3.0, 9.2)</td>
<td>3.27 (1H, s, NH)</td>
</tr>
</tbody>
</table>

Fig. 1. Structures of Compounds 1–2.
spectrum, assigned presence of two ortho-
protons pattern resonated at δH 5.69 (1H, d, J = 8.4 Hz) and 8.01 (1H, d, J = 8.4 Hz) was also observed in 1H NMR spectrum. The presence of four oxygenated methines at δH 4.01 to 5.90 and oxygenated methylene at δH 3.83 (J = 2.6, 9.8 Hz) and 3.73 (J = 3.3, 9.1 Hz), were assigned to ribose unit which formed by autohydrolysis reaction of the O-glycosyl saccharinic acid (Aspinal, 1976), suggesting the presence of saccharide component in 1.

A total twenty seven carbon resonances were observed in the 13C NMR spectrum. These were assigned by DEPT and HMBC experiments to two olefinic carbons at δC 102.7 and 142.8, one quartenary carbon, two amide carbonyl at δC 166.3 and 152.0, originating from lactam ring. The presence of four sp2 oxygenated methines carbon, nine sp2 nitrogenated methylenes were assigned to the presence of straight chain with amino hydroxychylalino formed by asparagine reaction in 1, which continued by hydrolysis reaction into aliphatic asparagine chain. In addition, four sp3 oxygenated methines together with one sp2 oxygenated methylene also observed in 13C NMR, suggested the presence of a ribose ring in 1. These functionalities accounted for three out of the total five degrees of unsaturation. The remaining two degrees of unsaturation were consistent to bisamide aliphatic asparagine chain derivative with a ribose and lactam ring.

A comparison of the NMR data of 1 with uridine (Jian et al., 2010), revealed that the structures of the two compounds are closely related in both lactam and ribose ring and different in aliphatic asparagine chain. Position of two amide carbonyl group at C-1′′′ and C-4′′′ together with ribose unit were confirmed on basis of HMBC and 1H-1H-COSY spectra (Fig. 2). The down field chemical shift of C-1′′′ at δH 90.7 was assigned for oxygenated methine which bonded with a nitrogen atom. The proton of H-1′′′ at δH 5.90, J = 4.5 Hz was correlated to C-2′′′ (δH 102.7), C-3′′′ (δH 142.8), and C-4′′′ (δH 152.0), suggested the presence of a ribose ring in lactam ring. The down field chemical shift of H-5′′′ at δH 3.83 and 3.73, was correlated to C-3′′′ (δC 71.4) and C-4′′′ (δC 86.4), and C-2′′′ (δC 75.9) through a long-range correlation, supported the presence of a ribose ring. The relative stereochemistry of 1, was established by coupling constant of proton H-1′′′ and H-2′′′ at δH 3.50 and 3.54, respectively with oxygenated methine at δH 4.14 (J = 5.2 Hz) identified that the straight-chain of compound 2 is straight-chain with amino hydroxychylalino. The Proton of H-2′′′ and H-3′′′ at δH 3.62 (J = 5.8,10.5 Hz) and H-1 at 4.14 (J = 5.2 Hz) were assigned for β-configuration. Therefore the chemical structure of 2 was established as new bisamide derivative was named as an eximiamide B.

![Fig. 2. Selected HMBC correlations for 1.](image-url)
The cytotoxicity effects of the two isolated compounds against the P-388 murine leukemia cells were conducted according to the method described in previous paper (Alley et al., 1998) and were used an artonin E (IC_{50} 0.3 μg/mL) as a positive control (Hakim et al., 2007).

Cytotoxic activity of eximiamide A (1) and eximiamide B (2), was influenced by the presence of hydroxyl, amide carbonyl groups (Harneti et al., 2014; Saifah et al., 1999), and amine functional group, was transformed to be quaternary system by protonation. Insignificant activity of compound 1 than 2, was influenced by folding process between straight-chain amino hydroxyethylamino with hydroxyl groups of 1, it can disturb the amine function transformation to be quaternary system. Unlike compound 1, folding process of 2 is not occur. These results suggested that a quaternary system moiety from amine function may be an important structural feature for cytotoxic activity in amide structures.

3. Experimental procedure

3.1. General

UV spectra were measured by using Shimazu UV–8452A with methanol. Optical rotations were recorded on an ATAGO AP-300 automatic polarimeter. The IR spectra were recorded on a Perkin-Elmer spectrum-100 FT-IR in KBr. Mass spectra were obtained with a Waters, Qtof HR-MS XEVeem mass spectrometer. NMR spectra were obtained with a JEOL JNM A-500 spectrometer using TMS as internal standard. Chromatographic separations were carried out on silica gel 60 (70–230 and 230–400 mesh, Merck), Octa Desyl Silane (200–400 mesh, Fuji Silysia), PTLC glass plates were precoated with silica gel GF254 (Merck, 0.25 mm). Chromatogram of HPLC were obtained with a Waters HPLC 1525 with carbohydrate analysis column, 3.9 x 300 mm. TLC plates were precoated with silica gel GF254 (Merck, 0.25 mm) and detection was achieved by spraying with 10% H₂SO₄ in ethanol, followed by heating.

3.2. Plant material

The bark of A. eximia were collected in Bogor Botanical Garden, Bogor, West Java Province, Indonesia in June 2011. The plant was identified by the staff of the Bogoriense Herbarium, Bogor, Indonesia and a voucher specimen (No. Bo-1295315) was deposited at the herbarium.

3.3. Extraction and isolation

Dried ground bark (4 kg) of A. eximia were extracted successively with n-hexane, EtOAc, and MeOH. Evaporation resulted in the crude extracts of n-hexane (26.4 g), EtOAc (54.5 g), and MeOH (32.5 g), respectively. The n-hexane, ethyl acetate and methanol extracts exhibited a cytotoxic activity against P-388 murine leukemia cells with IC_{50} values of 28, 58 and 40 μg/mL, respectively. The methanol extract (32.5 g) was subjected to vacuum liquid chromatography over silica gel using a gradient elution of mixture of CHCl₃/MeOH (10:0–0:10) as eluting solvents to afford 12 fraction (M01–M12). Fraction M05 (11 g) was subjected to column chromatography over silica gel using a gradient mixture of EtOAc/MeOH (10:0–4:1) as eluting solvents to afford 12 fraction (N01–N012). Fraction N03 (244.7 mg) was subjected to column chromatography over silica gel using a gradient mixture of CHCl₃/MeOH (10:0–4:1) as eluting solvents to afford 25 fraction (O01–O25). Fractions (O10–O15) were combined (25.4 mg) and was chromatographed on ODS using a gradient mixture of MeOH-H₂O to afford 5 fractions (P01–P05). Fraction P03 (18.5 mg) was preparative TLC on silica gel GF254, eluted with CHCl₃: MeOH (4:1), to give 1 (10.4 mg). Fractions (N05–N101) were combined (25.4 mg) and was chromatographed on ODS using a gradient mixture of MeOH-H₂O to afford 5 fractions (P01–P05). Fraction P03 (18.5 mg) was preparative TLC on silica gel GF254, eluted with CHCl₃: MeOH (4:1), to give 2 (5.0 mg).

3.3.1. Eximiamide A (1)

Yellow oil; [α]_D^20_0 = −10.5 (c, 0.1, MeOH); UV MeOH λ_max nm (log ε): 262 (5.25); IR (KBr) ν_max cm⁻¹: 3400, 2935, 1618, 1409, 1271, 1110; 1H NMR (CD₂OD, 500 MHz) see Table 1; 13C NMR (CD₂OD, 125 MHz), see Table 1; HR-TOFMS (positive ion mode) m/z 637.7206 (calcd. for C₂H₆N₆O₁₁ m/z 636.7354).

3.3.2. Eximiamide B (2)

Yellow oil; [α]_D^20_0 = 24.6 (c, 0.1, MeOH); UV MeOH λ_max nm (log ε): 268 (5.23); IR (KBr) ν_max cm⁻¹: 3409, 2935, 1678, 1411, 1271, 1110; 1H NMR (CD₂OD, 500 MHz) see Table 1; 13C NMR (CD₂OD, 125 MHz), see Table 1; HR-TOFMS (negative ion mode) m/z 416.4528 (calcd. for C₁₀H₁₂N₂O₂ m/z 417.4540).

3.3.3. Determination of sugar unit in 1

Compound 1 (2 mg) was hydrolysed with 2 N H₂SO₄ (2 mL) for 1 h at 50 °C. After cooling, the mixture was diluted with 5 mL water and extracted with CHCl₃. The aqueous layer was evaporated in reduced pressure to yield a brown residue. The residue was dissolved in MeOH and analyzed by HPLC. The sugar was identified as d-ribose (t_R 2.88 min) from the hydrolysis experiments with 1 [authentic samples: d-ribose (t_R 2.88 min)].

3.4. Determination of cytotoxic activity

The cytotoxic activity assay was conducted according to the method described by Alley et al. (1998). The P-388 cells were seeded into 96-well plates at an initial cell density of approximately 3.10^4 cells cm⁻². After 4 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 7.3–7.65). 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 by using a micro plate reader at 550 nm. IC₅₀ values were taken from the plotted graph of percentage live cells compared to control (%), receiving only PBS and DMSO, versus the tested concentration of compounds (μg/mL). The IC₅₀ value is the concentration required for 50% growth inhibition. Each assay and analysis was run in triplicate and averaged.

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