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Dammarane Triterpenoids from *Aglaia eximia* Miq. and Their Cytotoxic Activity Against P388 Murine Leukemia Cell

Kindi Farabi[1], Desi Harneti[1], Nurlelasari[1], Rani Maharani[1,2], Tri Mayanti[1], Ace Tatang Hidayat[1], Sofa Fajriah[3], Al Arofatus Naini[3], Ferry Ferdiansyah Sofian[4], Mohamad Nurul Azmi[5], Unang Supratman[1,2*]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor 45363, Indonesia
[2]Central Laboratory of Universitas Padjadjaran, Jatinangor 45363, Indonesia
[3]Research Center for Pharmaceutical Ingredients and Traditional Medicine, National Research and Innovation Agency (BRIN), Cibinong Science Center Complex, Cibinong 16911, West Java, Indonesia
[4]Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Padjadjaran, Jatinangor, Sumedang 45363, Indonesia
[5]School of Chemical Sciences, Universiti Sains Malaysia,11800 Minden, Penang, Malaysia

*Corresponding author email: unang.supratman@unpad.ac.id

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ABSTRACT. Triterpenoids are a large group of secondary metabolites commonly found in plants, exhibiting high diversity in both structural features and biological activities. *Meliaceae* family is knows as a rich source of the triterpenoid compounds. As the most extensive genus within the *Meliaceae* family, *Aglaia* is known to contain many bioactive triterpenoid compounds, including cytotoxic triterpenoids. Among the various types of triterpenoids, dammarane is frequently found in *Aglaia* and has demonstrated potential cytotoxic activity. This purpose of the research was to isolate and structure determination of four dammarane triterpenoids from the methanol extract of *Aglaia eximia* stem bark. All four compounds were successfully isolated and identified as, dammar-24-en-3α,20-diol (**1**), 20*S*,24*S*-epoxy-dammar-3α,25-diol (**2**), (*E*)-dammar-23-en-3α,20,25-triol (**3**), and (*E*)-25-hydroperoxydammar-23-en-3α,20-diol (**4**), respectively. The compounds were analyzed using a combination of spectroscopic techniques, including HRMS (high-resolution mass spectroscopy), FTIR (fourier transform infrared spectroscopy), and NMR (nuclear magnetic resonance, one and two dimensional). Cytotoxicity assays using the MTT method were applied to compounds **1**-**4**. All isolated compounds (**1**-**4**) generated moderate cytotoxic activity against P388 murine leukemia cells with IC_{50} 9.09, 11.03, 5.89, and 5.74 µg/mL, respectively. Preliminary structure-activity relationship (SAR) analysis suggested that the presence of hydroxyl and hydroperoxyl groups at C-25 increases cytotoxicity, whereas the cyclization in the side chain to form an epoxide ring decreases cytotoxicity.

Keyword: Triterpenoids, *Meliaceae*, *Aglaia eximia*, cytotoxicity, P388 murine leukemia cells.

INTRODUCTION

Triterpenoids are a varied group of plant compounds, typically consisting of about 30 carbon atoms linked together from 6 isoprene units (Hill & Connolly, 2020; Ludwiczuk et al., 2017). These compounds are commonly found across different plants, either in their natural, unaltered form or as conjugates bonded with sugar groups or other molecules. This combination can create specialized metabolites with increased biological activity (Cárdenas et al., 2019).

Triterpenoids exhibit potential bioactivity, mainly caused by its highly diverse chemical structure. These bioactivities include antiviral, analgesic, anti-inflammatory, antimicrobial, anti-ulcer, and anti-cancer effects (Yasin et al., 2021; Borella et al., 2019). Their primary application lies in their cytotoxic properties (Noushahi et al., 2022). For example, triterpenoids from *Ganoderma lucidum* have shown anticancer properties, which are attributed to the highly oxidized lanostanes they contain (Qu et al., 2017). In China, ginseng is considered a significant traditional medicinal plant, known for its triterpene saponins called ginsenosides (Kim, 2018). Thus, there is no doubt that triterpenoids are a vital source of cytotoxic compounds.

Aglaia is recognized as the largest genus within the *Meliaceae* family, found across tropical and subtropical regions of Asia such as southeast Asia, northern parts of Australia, and the Pacific island (Pan et al., 2014). In Indonesia, 65 species out of 150 species are grown (Harneti and Supratman, 2021). About more than 100 triterpenoid compounds have been isolated from this genus (Harneti & Supratman,

2021; Farabi et al., 2022a and 2022b). The biological activities of these plants have been studied, including insecticidal, antibacterial, antitumor, antiviral, antifungal, and cytotoxic activities (Harneti & Supratman, 2021; Farabi et al., 2017; Hidayat et al., 2018).

Aglaia eximia is a plant mainly located at southern Sumatra Island in Indonesia. Traditionally, its bark has been utilized in Indonesian folk medicine to alleviate fever, hydrate the lungs, and treat contusions, coughs, and skin conditions (Harneti et al., 2014). Previous investigation on this species led to the isolation of new stigmastane steroid (Harneti et al., 2014), flavonoid glycosides (Sianturi et al., 2016a), bisamide (Sianturi et al., 2015), and lignan (Sianturi et al., 2016b). However, there are the limited number of triterpenoid compounds reported in this species, thus, this research is interesting for revealing its triterpenoid content. In this study, four dammarane triterpenoid compounds (**1**-**4**) (**Figure 1**) were isolated and identified for the first time in this species. The compounds were separated and purified using chromatographic techniques and crystallization. Additionally, their cytotoxicity was tested against P388 murine leukemia cells.

EXPERIMENTAL SECTION

General Experiment Procedure

The FTIR spectra were obtained on a Perkin-Elmer FT-IR using KBr. NMR data were recorded on a JEOL NMR spectrometer 500 MHz for ^{1}H spectra and 125 MHz for ^{13}C spectra with tetramethylsilane (TMS) as an internal standard. Mass Spectra were obtained on Waters Q-TOF Xevo mass spectrometer. Silica gel (70-230 mesh and 230-400 Mesh, Merck) and ODS 100–200 mesh (Fuji Sylisia Chemical LTD) were used for purification with column chromatography (CC). TLC was carried out with silica gel GF_{254} and RP-18 F_{254} plates (Merck, 0.25 mm). TLC visualization was conducted by spraying the plates with a 10% H_2SO_4 solution in ethanol, followed by heating at 120 °C.

Plant Material

In June 2011, *A. eximia* stembark was collected from the Botanical Garden in Bogor. The plant was unambiguously identified as *A. eximia* by personnel at the Bogoriense Herbarium in Bogor, Indonesia, with specimen No. Bo-1295315.

Extraction and Isolation

The stem bark of *A. eximia* was dried at room temperature to achieve 3.4 kg of *A. eximia* stembark (3.4 kg). The methanol was used for the maceration of *A. eximia* stembark for six days (6 x 10 L). After filtration, the methanol extract was concentrated under reduced pressure using a rotary evaporator, yielding 320 g of brown condensed extract. This concentrated extract was then partitioned using *n*-hexane, ethyl acetate, and *n*-butanol. After further evaporation under reduced pressure, 32 g of *n*-hexane extract, 67 g of ethyl acetate extract, and 42 g of *n*-butanol extract were obtained.

The *n*-hexane extract was fractionated using vacuum liquid chromatography (VLC) on silica gel 60 with a stepwise gradient elution of n-hexane, ethyl acetate, and methanol, each increasing by 10%. This process resulted in ten distinct fractions labeled A through J. Fraction B (1.8 g) was further divided using column chromatography on silica gel (70-230 mesh) with a 2.5% gradient of *n*-hexane and ethyl acetate, yielding seven fractions (B1-B7). Subsequently, Fraction B2 (230 mg) was subjected to column chromatography on ODS (100-200 mesh) with an isocratic elution of methanol and water in a 9.5:0.5 ratio, producing four fractions (B2a-B2d). Compound **1** (6 mg) was isolated by crystallizing fraction B2c. Fraction B4 (320 mg) was separated using column chromatography on silica gel (70-230 mesh) with an isocratic elution of *n*-hexane and ethyl acetate in an 8:2 ratio, resulting in five fractions (B4a-B4e). Crystallization of fraction B4b yielded compound **2** (11 mg). Fraction C (2.3 g) was divided using column chromatography on silica gel (70-230 mesh) with a

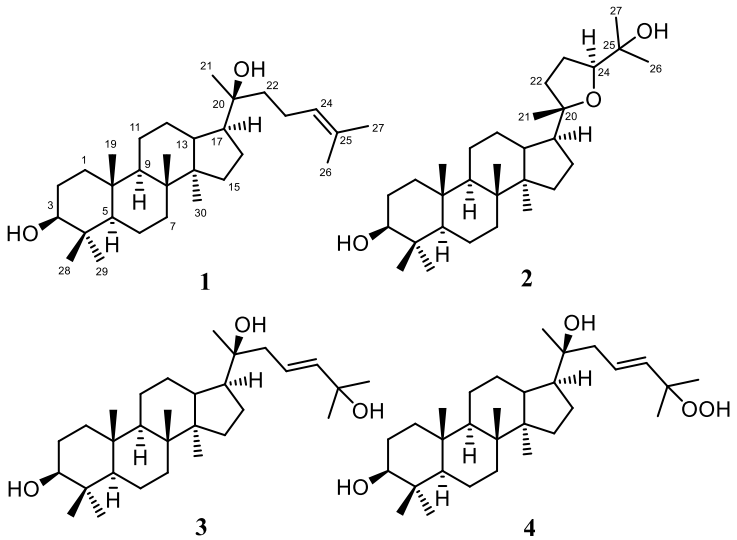

Figure 1. Chemical structure of compounds **1**-**4**.

2.5% gradient of *n*-hexane and acetone, resulting in nine fractions (C1-C9). Fraction C1 (78 mg) was further separated using column chromatography on ODS (100-200 mesh) with an isocratic elution of methanol and water in a 4:1 ratio, yielding three fractions (C1a-C1c). Compound **3** (7 mg) was obtained by crystallizing fraction C1c. Fraction C2 (503 mg) was separated using column chromatography on silica gel (70-230 mesh) with a 1% gradient of *n*-hexane and ethyl acetate, producing seven fractions (C2a-C2g). Crystallization of fraction C2b yielded compound **4** (4 mg).

Compound **1** was obtained as white amorphous powder. IR (KBr) υ_{max} 3353, 2940, 2868, 1449, 1378, 1069 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 1.25 (1H, m, H-1a), 1.44 (1H, m, H-1b), 1.53 (1H, m, H-2a), 1.94 (1H, m, H-2b), 3.39 (1H, t, *J*=4.7 Hz, H-3), 1.25 (1H, m, H-5), 1.44 (2H, m, H-6), 1.53 (2H, m, H-7), 1.44 (1H, m, H-9), 1.56 (2H, m, H-11), 1.56 (1H, m, H-12a), 1.94 (1H, m, H-12b), 1.56 (1H, m, H-13), 1.25 (2H, m, H-15), 1.76 (2H, m, H-16), 1.76 (2H, m, H-17), 0.95 (3H, s, CH_3-18), 0.85 (3H, s, CH_3-19), 1.13 (3H, s, CH_3-21), 1.44 (2H, m, H-22), 2.03 (2H, dd, *J*=7.1 & 14.9 Hz, H-23), 5.11 (1H, t, *J*=7.1 Hz, H-24), 1.68 (3H, s, CH_3-26), 1.61 (3H, s, CH_3-27), 0.93 (3H, s, CH_3-28), 0.83 (3H, s, CH_3-29), 0.88 (3H, s, CH_3-30); ^{13}C-NMR ($CDCl_3$, 125 MHz), see **Table 1**. HR-TOF-MS *m/z* 467.3848 $[M+Na]^+$ (Calcd. $C_{30}H_{52}O_2Na^+$, *m/z* 467.3865).

Compound **2** was obtained as white amorphous powder. IR (KBr) υ_{max} 3487, 2960, 2845, 1447, 1375, 1093 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 1.42 (2H, m, H-1), 1.55 (2H, m, H-2), 4.60 (1H, t, *J*=3.1 Hz, H-3), 1.24 (1H, m, H-5), 1.39 (2H, m, H-6), 1.63 (2H, m, H-7), 1.44 (1H, m, H-9), 1.53 (2H, m, H-11), 1.75 (2H, m, H-12), 1.62 (1H, m, H-13), 1.04 (2H, m, H-15), 1.51 (2H, m, H-16), 1.44 (1H, m, 17), 0.85 (3H, s, CH_3-18), 0.88 (3H, s, CH_3-19), 1.13 (3H, s, CH_3-21), 1.23 (2H, m, H-22), 1.84 (2H, m, H-23), 3.61 (1H, dd, *J*=5.0 & 10.2 Hz, H-24), 1.18 (3H, s, CH_3-26), 1.07 (3H, s, CH_3-27), 0.93 (3H, s, CH_3-28), 0.83 (3H, s, CH_3-29), 0.94 (3H, s, 30); ^{13}C-NMR ($CDCl_3$, 125 MHz), see **Table 1**. HR-TOF-MS *m/z* 461.3993 $[M+H]^+$ (Calcd. $C_{30}H_{53}O_3^+$, *m/z* 461.3995).

Compound **3** was obtained as white amorphous powder. IR (KBr) υ_{max} 3436, 2945, 2873, 1456, 1380, 1074 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 1.25 (1H, m, H-1a), 1.43 (1H, m, H-1b), 1.72 (1H, m, H-2a), 1.95 (1H, m, H-2b), 3.39 (1H, t, *J*=2.6 Hz, H-3), 1.25 (1H, m, H-5), 1.43 (2H, m, H-6), 1.56 (2H, m, H-7), 1.43 (1H, m, H-9), 1.56 (2H, m, H-11), 1.56 (1H, m, H-12a), 1.94 (1H, m, H-12b), 1.56 (1H, m, H-13), 1.05 (2H, m, H-15), 1.84 (2H, m, H-16), 1.73 (1H, m, H-17), 0.96 (3H, s, CH_3-18), 0.85 (3H, s, CH_3-19), 1.12 (3H, s, CH_3-21), 2.19 (2H, m, H-22), 5.69 (1H, m, H-23), 5.69 (1H, m, H-24), 1.32 (3H, s, CH_3-26), 1.32 (3H, s, CH_3-27), 0.93 (3H, s, CH_3-28), 0.85 (3H, s, CH_3-29), 0.85 (3H, s, CH_3-30); ^{13}C-NMR ($CDCl_3$, 125 MHz), see **Table 1**. HR-TOF-MS *m/z* 461.3946 $[M+H]^+$ (Calcd. $C_{30}H_{53}O_3^+$, *m/z* 461.3989).

Compound **4** was obtained as a white amorphous powder. IR (KBr) υ_{max} 3446, 2943, 2880, 1458, 1376, 1070, 991 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 1.69 (2H, dd, *J*=3.5 & 13.4 Hz, H-1), 1.46 (1H, m, H-2a), 1.70 (1H, m, H-2b), 3.20 (1H, dd, *J*=4.7 & 11.5 Hz, H-3), 0.72 (1H, dd, *J*=2.3 & 9.5 Hz, H-5), 1.41 (1H, m, H-6a), 1.55 (1H, m, H-6b), 1.27 (2H, m, H-7), 1.29 (1H, m, H-9), 1.22 (2H, m, H-11a), 1.48 (1H, m, H-11b), 1.59 (2H, m, H-12), 1.63 (1H, m, H-13), 1.08 (1H, dd, *J*=1.7 & 8.5 Hz, H-15a), 1.45 (1H, m, H-15b), 1.22 (1H, m, H-16a), 1.81 (1H, m, H-16b), 1.73 (1H, dd, *J*=3.4 & 6.8 Hz, H-17), 0.93 (3H, s, CH_3-18), 0.82 (3H, s, CH_3-19), 1.13 (3H, s, CH_3-21), 2.25 (2H, dd, *J*=7.7 & 11.3 Hz, H-22), 5.77 (1H, dd, *J*=7.7 & 16.1 Hz, H-23), 5.61 (1H, dd, *J*=4.7 & 16.1 Hz, H-24), 1.33 (3H, s, CH_3-26), 1.34 (3H, s, CH_3-27), 0.97 (3H, s, CH_3-28), 0.75 (3H, s, CH_3-29), 0.85 (3H, s, CH_3-30); ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 1. HR-TOF-MS *m/z* 477.3951 $[M+H]^+$ (Calcd. $C_{30}H_{53}O_4^+$, *m/z* 477.3938).

Determination of Cytotoxic Activity

The MTT assay was utilized for the cytotoxic assay, following the procedures outlined by Farabi et al., 2018, with artonin E as the positive control (Hakim et al., 2017). P388 murine leukemia cells were placed in 96-well plates at an initial density of about 3 x 10^4 cells/cm^2. Following a 24-hour incubation period to enable cell attachment and growth, different concentrations of the samples were introduced. The compounds in DMSO were introduced at specific concentrations and six additional concentrations were made using a phosphate-buffered saline (PBS) solution (pH 7.30-7.65). The negative control consisted of DMSO alone. After incubating for 48 hours, the MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] was added and the incubation continued for an additional 4 hours. A stop solution with SDS (sodium dodecyl sulfate) was then introduced, and the incubation was extended for an additional 24 hours. The optical density was assessed at 550 nm using a microplate reader. The percentage of viable cells compared to the control (PBS and DMSO only) was graphed against the compound concentrations to calculate the IC_{50} values in μg/mL, which indicate the concentration needed to reduce cell growth by 50%. Each experiment was conducted in triplicate, and the results were averaged.

RESULTS AND DISCUSSION

The isolation process involved multiple stages using chromatographic techniques such as vacuum liquid chromatography (VLC) and open column chromatography, resulting in four dammarane triterpenoid compounds (labeled as **1**-**4** in **Figure 1**). The structures of these compounds were elucidated through comprehensive spectroscopic analysis,

incorporating one and two dimensional NMR (^{1}H-NMR, ^{13}C-NMR, DEPT, HMQC, HMBC, and ^{1}H-^{1}H COSY).

The extensive chromatography and recrystallization resulted a white amorphous powder of compound **1**. Chemical formula of compound **1** identified as $C_{30}H_{52}O_2$, with an ion peak in HRMS at *m/z* 467.3848 $[M+Na]^+$ (calculated as 467.3865 for $C_{30}H_{52}O_2Na^+$). Functional group which existed in compound **1** were O-H (3353 cm^{-1}), C-H *sp*^{2} (2940 cm^{-1}), *gem*-dimethyl (1449 and 1378 cm^{-1}), and ether (1069 cm^{-1}) groups according to FTIR spectrum. The carefully observation of ^{1}H NMR spectrum demonstrated eight tertiary methyl groups (δ_H 0.83, 0.85, 0.88, 0.93, 0.95, 1.13, 1.61, and 1.68, each 3H), one oxymethine proton at δ_H 3.39 (1H, t, *J*=2.7 Hz), and one *sp*^{2} methine proton at δ_H 5.11 (1H, t, *J*=7.1 Hz). The amount of these methyl groups is suitable for dammarane triterpenoid compounds (Farabi et al., 2022). The ^{13}C NMR spectrum (**Table 1**) showed 30 distinct carbon signals. The DEPT experiment identified these as eight methyls, ten methylenes, six methines (one of them is one *sp*^{2} methine at δ_C 124.9 and one oxymethine at δ_C 76.4), and six quaternary carbons (incorporating one oxygenated quaternary carbon at δ_C 75.6 and one *sp*^{2} quaternary carbon at δ_C 131.7). The alkene functional groups accounted for one out of five degrees of unsaturation, with the residual four degrees were attributed to a tetracyclic dammarane triterpenoid skeleton. The positioning of functional groups and the main skeleton of compound **1** were confirmed through HMBC and ^{1}H-^{1}H COSY (**Figure 2**). HMBC correlations of each methyl group with adjacent carbons confirmed the dammarane triterpenoid structure. Specific correlations, such as those of H-28, H-29 to C-3, C-4, and C-5, and of H-1 to C-3, confirmed the hydroxyl group at C-3. Another hydroxyl group was attached at C-20 according to HMBC cross peak of H-21 and H-22 to C-20. The correlations of H-26 and H-27 to C-24 and C-25 was unambiguously proved that the double bond formed at C-24/C-25. This resulted also supported by ^{1}H-^{1}H COSY which confirmed the double bond at C-24/C-25 from correlations of H-22/H-23/H-24. The NMR data of compound **1** was carefully compared with dammar-24-en-3β,20-diol obtained from the stem bark of *Aglaia elliptica* (Hidayat et al., 2017) and the result showed that two compounds were performed high similarity. Thus, compound **1** was identified first time from *A. eximia* and based on all spectroscopic evidenced determined as dammar-24-en-3β,20-diol.

The extensive chromatography and recrystallization resulted a white amorphous powder of compound **2**. Chemical formula of compound **2** identified as $C_{30}H_{52}O_3$ based on HRMS with *m/z* ion peak at 461.3993 $[M+H]^+$ (calcd. for $C_{30}H_{53}O_3^+$, *m/z* 461.3995). The FTIR spectra revealed the existances of O-H (3487 cm^{-1}), C-H *sp*^{2} (2960 cm^{-1}), *gem*-dimethyl (1447 and 1375 cm^{-1}), and ether (1093 cm^{-1}) groups. The ^{1}H NMR spectra observed the appearance of resonating eight tertiary methyls (δ_H 0.83, 0.85, 0.88, 0.93, 0.94, 1.07, 1.13, and 1.18, each 3H) and two oxymethine at δ_H 3.38 (1H, t, *J*=3.1 Hz) and 3.61 (1H, dd, *J*=5.0, 10.2 Hz). The number of methyl groups indicating the characteristic of triterpenoid core structure (Farabi et al., 2022). The total 30 signal were resonating in ^{13}C NMR spectra (**Table 1**). The type of carbon also clarified by the DEPT experiment as eight methyls, ten methylenes, six methines (including two methines at δ_C 76.4 and 86.3), and six quaternary carbons (including two oxygenated quaternary carbons at δ_C 70.3 and 86.7). The five distinct levels of unsaturation were found to be consistent with the following a tetracyclic core in dammarane triterpenoid with additional of epoxide ring in side chain. The position of each functional group including its main skeleton of compound **2** were deduced based on HMBC and ^{1}H-^{1}H COSY cross peak (**Figure 2**). The HMBC of each methyl group to corresponding neighbour carbons clarified the main core of dammarane triterpenoid structure. Cross peak of H-28, H-29 to C-3, C-4, and C-5 and cross peak of H-1 to C-3 confirmed the attachment of hydroxyl group at C-3. Another hydroxyl group was determined attach at C-25 based on correlation of H-26, H-27 to C-25. The position of epoxide cyclic was identified at C-20/C-24 according to cross peak of H-24 to C-20 and H-21 to C-20, C-22, and C-17. The ^{1}H-^{1}H COSY confirmed the position of hydroxyl group at C-3 from the cross peak of H-1/H-2/H-3. Spectroscopic data comparison, especially NMR data of compound **2** with 20*S*,24*S*-epoxy-dammar-3β,25-diol or 3β-ocotillol isolated from the *Aglaia elliptica* stembark (Hidayat et al., 2017) revealed that those two compounds were identical. Thus, compound **2** present as 20*S*,24*S*-epoxy-dammar-3β,25-diol which first time isolated from *A. eximia*.

The extensive chromatography and recrystallization resulted a white amorphous powder of compound **3**. Chemical formula of compound **3** identified as $C_{30}H_{52}O_3$ at *m/z* ion peak 461.3946 $[M+H]^+$ (calculated as 461.3989 for $C_{30}H_{53}O_3^+$) in HRMS spectrum. The FTIR spectrum indicated the presence of O-H (3436 cm^{-1}), C-H *sp*^{2} (2945 cm^{-1}), gem-dimethyl (1456 and 1380 cm^{-1}), and ether (1074 cm^{-1}) groups. The ^{1}H NMR spectrum signals implied the characteristic of eight tertiary methyl groups (δ_H 0.85, 0.85, 0.85, 0.93, 0.96, 1.12, 1.32, and 1.32, each 3H), one oxymethine at δ_H 3.39 (1H, t, *J*=2.6 Hz), and two *sp*^{2} methines at δ_H 5.69 (2H, m). These methyl groups are characteristic of triterpenoid compounds (Farabi et al., 2022). The ^{13}C NMR spectrum (**Table 1**) revealed 30 distinct carbon signals. DEPT analysis further identified these as eight methyls, nine methylenes, seven methines (one the them was two *sp*^{2} methines at δ_C 122.5 and 142.1, and one oxymethine

at δ_C 76.4), and six quaternary carbons (including two oxygenated quaternary carbons at δ_C 75.2 and 70.9). The functional groups were found to account for one in five degrees of unsaturation, with the other four degrees of unsaturation being accounted for a tetracyclic dammarane triterpenoid core. A detailed comparison of the NMR data for compound **3** with that of (*E*)-dammar-23-en-3β,20,25-triol, previously isolated from the hypocotyls and fruits of *Ceriops tagal* (Pakhathirathien et al., 2005), showed that those compounds were identical. Therefore, compound **3** was recognized as (*E*)-dammar-23-en-3β,20,25-triol, marking its first isolation from *A. eximia*.

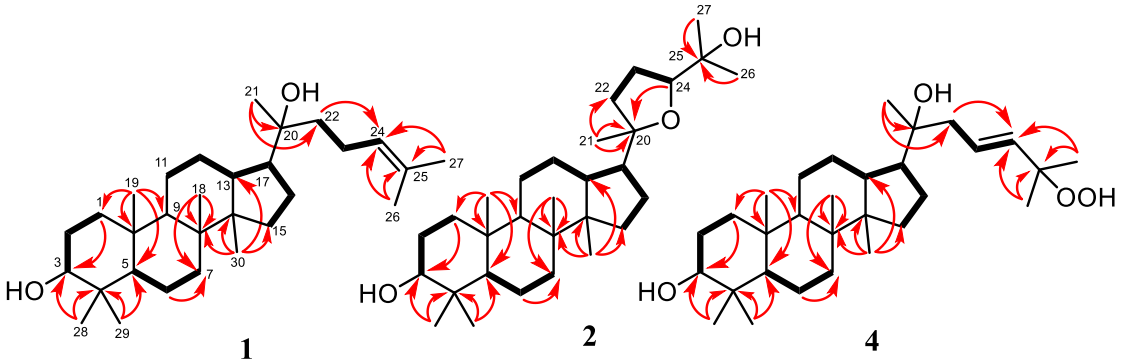

Figure 2. Correlations of HMBC and ^{1}H–^{1}H COSY for compounds **1**, **2**, and **4**.

Tabel 1. ^{13}C NMR (125 MHz in $CDCl_3$) data for compounds **1**-**4**.

No.	Compound			
	1	**2**	**3**	**4**
	δc (mult.)	δc (mult.)	δc (mult.)	δc (mult.)
1	33.8 (t)	33.7 (t)	33.8 (t)	39.2 (t)
2	24.9 (t)	25.4 (t)	24.9 (t)	24.8 (t)
3	76.4(d)	76.4 (d)	76.4 (d)	79.2 (d)
4	37.8 (s)	37.3 (s)	37.8 (s)	39.1 (s)
5	49.7 (d)	49.6 (d)	49.7 (d)	55.8 (d)
6	18.4 (t)	18.3 (t)	18.4 (t)	18.4 (t)
7	35.3 (t)	34.8 (t)	35.3 (t)	35.4 (t)
8	40.7 (s)	40.7 (s)	40.7 (s)	40.5 (s)
9	49.9 (d)	50.7 (d)	50.6 (d)	50.8 (d)
10	37.4 (d)	37.7 (s)	37.4 (s)	37.3 (s)
11	21.5 (t)	21.7 (t)	21.5 (t)	21.5 (t)
12	25.5 (t)	27.1 (t)	25.5 (t)	27.6 (t)
13	42.4 (d)	42.8 (d)	42.5 (d)	42.6 (d)
14	50.3 (s)	50.2 (s)	40.7 (s)	50.5 (s)
15	31.3 (t)	31.5 (t)	31.2 (t)	31.3 (t)
16	27.7 (t)	25.9 (t)	27.7 (t)	27.7 (t)
17	50.6 (d)	49.8 (d)	50.0 (d)	50.4 (d)
18	16.2 (q)	16.2 (q)	15.7 (q)	15.7 (q)
19	15.6 (q)	16.6 (q)	16.2 (q)	16.6 (q)
20	75.6 (s)	86.7 (s)	75.2 (s)	75.3 (s)
21	25.5 (q)	27.3 (q)	25.9 (q)	25.9 (q)
22	40.7 (t)	35.3 (t)	43.6 (t)	43.3 (t)
23	22.7 (t)	26.4 (t)	122.5 (d)	127.5 (d)
24	124.9 (d)	86.3 (d)	142.1 (d)	137.5 (d)
25	131.7 (s)	70.3 (s)	70.9 (s)	82.1 (s)
26	25.9 (q)	27.9 (q)	30.0 (q)	24.1 (q)
27	17.8 (q)	24.1 (q)	30.1 (q)	24.4 (q)
28	28.5 (q)	28.4 (q)	28.4 (q)	28.2 (q)
29	22.2 (q)	22.2 (q)	22.2 (q)	15.5 (q)
30	16.7 (q)	15.6 (q)	16.7 (q)	16.2 (q)

Table 2. Cytotoxic activities against P388 cell lines for **1**-**4**.

Compounds	IC_{50} (μg/mL)
dammar-24-en-3α,20-diol (**1**)	9.09
20*S*,24*S*-epoxy-dammar-3α,25-diol (**2**)	11.03
(*E*)-dammar-23-en-3α,20,25-triol (**3**)	5.89
(*E*)-25-hydroperoxydammar-23-en-3α,20-diol (**4**)	5.74
Artonin E*	0.03

*positive control

The extensive chromatography and recrystallization resulted a white amorphous powder of compound **4**. Chemical formula of compound **4** identified as $C_{30}H_{52}O_4$ with ion peak at *m/z* 477.3951 $[M+H]^+$ (calcd. for $C_{30}H_{53}O_4^+$, *m/z* 477.3938) based on HRMS spectrum. The FTIR, ^{1}H NMR and ^{13}C NMR spectra (**Table 2**) of compound **4** showed high similarity with compound **3** (**Table 1**). The main skeleton is identical as dammarane triterpenoids. However, the main difference of compound **3** and **4** was in functional group in C-25 position. The quaternary carbon at C-25 at compound **3** was resonated at δ_C 70.9, whereas at compound **4** was 82.1. This main difference suggested that at C-25, compound **4** attached an hydroperoxyl group instead of hydroxyl group which increased chemical shift of C-25. The presences of hydroperoxyl group at C-25 also proofed by the HMBC cross peaks of H-26, H-27 to C-24, C-25 (**Figure 2**). Finally, the spectroscopic data, especially from NMR of compound **4** with (*E*)-25-hydroperoxydammar-23-en-3β,20-diol isolated from the stembark of *Aglaia elliptica* (Hidayat et al., 2017) compared and revealed that two compounds were identical. Thus, compound **4** identified as (*E*)-25-hydroperoxydammar-23-en-3β,20-diol which first time isolated from *A. eximia*.

All isolated triterpenoid compounds (**1**-**4**) were tested against P388 murine leukemia cell line according to previously reported by Farabi et al., (2017) and artonin E used as a positive control. The resulted of IC_{50} appears in **Table 2**. According to US National Cancer Institute, all isolated compound classified as strong activity (range for strong activity is < 20 μg/mL) (Boik, 2001). However, the formation of epoxide ring at side chain at compound **2** decreased cytotoxic activity. Whereas, the presences of hydroxyl and hydroperoxyl group at C-25 (in compound **3** and **4**, respectively) increase cytotoxic activity. This result indicated that cyclisation in side chain will decreased cytotoxic activity of dammarane triterpenoid, in other hand, the additional polar functional group such as hydroxyl and hydroperoxyl groups increased cytotoxic activity.

CONCLUSIONS

Four dammarane triterpenoids have been isolated from *A. eximia* stembark, namely,(-)-dammar-24-en-3α,20-diol (**1**), 20*S*,24*S*-epoxy-dammar-3α,25-diol (**2**), (*E*)-dammar-23-en-3α,20,25-triol (**3**), and (*E*)-25-hydroperoxydammar-23-en-3α,20-diol (**4**) for the first time. Based on this study, dammarane triterpenoid with additional epoxide ring at side chain gave worse cytotoxicity than dammarane triterpenoid with no epoxide ring against P388 murine leukemia cell. The presence of polar functional groups (hydroxyl or hydroperoxyl) in side chain especially in C-25 suggested to rise the cytotoxic activity.

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