Cytotoxic Triterpenoids from the Stembark of *Aglaia argentea* (Meliaceae)

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ABSTRACT

*Four dammarane-type triterpenoid compounds, dammar-24-en-3α-ol (**1**), 3-epi-cabraleahydroxy lactone (**2**), (E)-25-hydroperoxydammar-23-en-3β,20-diol (**3**), and dammar-24-en-3β,20-diol (**4**), were isolated from the methanolic extract of the stembark of* Aglaia argentea*. Compounds, **1**-**4**, were isolated for first time from this plant. The structure of isolated compounds were elucidated by spectroscopic methods including one and two-dimensional NMR as well as mass spectrometric analysis. Compounds **1**-**4**, along with a known synthetic analog, 20-hydroxy-dammar-24-en-3-on (**5**), were evaluated their cytotoxic activity against P-388 murine leukemia cells in vitro. The IC_{50} values of compounds, **1**-**5** were 9.09 ± 0.10, 68.53 ± 0.08, 5.89 ± 0.08, 22.40 ± 0.11, and 11.53 ± 0.08 µg/mL, respectively. Among the dammarane-type triterpenoids, compounds **1**, **3**, **4** and **5** having opened side chain showed the stronger activity, where's compound **2** with a cyclic side chain showed weak or no activity. In addition, compound **3** showed the strongest activity, indicate that hydroperoxy group at side chain increase cytotoxic activity.*

Keywords: Aglaia argentea*; cytotoxic activity; dammarane-type triterpenoids; P-388 murine leukemia*

ABSTRAK

*Empat senyawa triterpenoid tipe damaran, damar-24-en-3α-ol (**1**), 3-epi-kabraleahidroksi lakton (**2**), (E)-25-hidroksi-damar-23-en-3β,20-diol (**3**), dan damar-24-en-3β,20-diol (**4**), telah disolasi dari ekstrak metanol dari kulit batang* Aglaia argentea*. Senyawa **1**-**4**, diisolasi untuk pertama kali dari tumbuhan ini. Senyawa **1**-**4**, bersama dengan senyawa analog sintetik yang telah dikenal, dammar-24-en,20-ol-3-on (**5**), dievaluasi aktivitas sitotoksiknya terhadap sel murin leukemia P-388 secara in vitro. Nilai IC_{50} senyawa **1**-**5** berturut-turut adalah 9,09 ± 0,10, 68,53 ± 0,08, 5,89 ± 0,08, 22,40 ± 0,11, dan 11,53 ± 0,08 µg/mL. Diantara senyawa triterpenoid tipe damaran, senyawa **1**, **3**, **4**, dan **5** yang memiliki rantai samping terbuka menunjukkan aktivitas lebih kuat, sementara senyawa **2** dengan rantai samping siklik menunjukkan aktivitas lemah atau tidak aktif. Sebagai tambahan, senyawa **3** menunjukkan aktivitas sitotoksik paling kuat, mengindikasikan bahwa gugus hidroperoksi pada rantai samping meningkatkan aktivitas sitotoksik.*

Kata Kunci: Aglaia argentea*; aktivitas sitotoksik; triterpenoid tipe damaran; sel murin leukemia P-388*

INTRODUCTION

Dammarane-type triterpenoids have been gaining worldwide attention for a long time because of their potent bioactivities [1]. Dammarane-type triterpenoids are distributed in genera of *Panax* (Araliaceae), *Gynostemma* (Cucurbitaceae), *Aralia* (Araliaceae), *Aglaia* (Meliaceae), *Bacopa* (Scrophulariaceae), *Copernicia* (Arecaceae), *Celastrus* (Celastraceae), *Forsythia* (Oleaceae), *Myrica* (Myrica), *Rhus* (Anacardiaceae), *Polyscias* (Araliaceae) and *Sapindus* (Sapindus) [2-6]. The basic molecular skeleton of dammarane-type triterpenoids comprises tetracyclic moiety and side chain moiety [1]. The bioactivities and chemical properties of dammarane-type triterpenoids have been investigated, and several pharmacological effects have been disclosed, including anti-fatigue, antihyperglycemic, antiobesity, anticancer, anti-HIV, antioxidant, antiaging, immunostimulatory, antiatherosclerotic and antihypertensive effects [7-9]. 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 [10]. The genus of

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Aglaia traditionally used to moisturizing the lungs, reducing fever and for treating the contused wound, coughs and skin diseases [11]. In Indonesia, flowers of *A. odorata* traditionally used as an insect repellent [12].

Some species of *Aglaia* have been phytochemically investigated previously with unique biological activity, such as antifungal sesquiterpenoids [13-14], diterpenoids [15-16], insecticidal and cytotoxic rocaglate derivatives [17-18], lignans [19-20], cytotoxic and antiviral dammarane-type triterpenoids [21,24] and antitumor cycloartane-type triterpenoids [4-5,8].

In our continuous search for cytotoxic constituents against P-388 murine leukemia cells from Indonesian *Aglaia* plants, we isolated and described two new cytotoxic triterpenoids, aglinone, and aglinin E, from the bark of *A. smithii* [23], a new stigmastane steroid, 3,4-epoxy-(22*R*,25)-tetrahydrofuran-stigmast-5-en, two new bisamide compounds, eximiamide A and B, from the bark of *A. eximia* [24-25] and one lignan [20].

In the further screening for cytotoxic compounds from Indonesia *Aglaia* plants, we found that the *n*-hexane and ethyl acetate extract of *A. argentea* exhibited a cytotoxic activity against P-388 murine leukemia cells with IC_{50} values of 26.72 and 15.49 µg/mL, respectively. We report herein the isolation, structural elucidation of triterpenoid compounds (**1**-**4**) and a synthetic analogue, **5**, together with their cytotoxic activity.

EXPERIMENTAL SECTION

Materials

The bark of *A. argentea* was 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.

Instrumentation

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 ^{1}H and 150 MHz for ^{13}C and 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 GF_{254} (Merck, 0.25 mm) and detection was achieved by spraying with 10% H_2SO_4 in EtOH, followed by heating.

Procedure

Extraction and isolation

The dried bark (2.5 kg) of *A. argentea* was extracted with methanol exhaustively (12 L) at room temperature for 5 days. After removal of the solvent under vacuum, the viscous concentrated of MeOH extract (133.5 g) was first suspended in H_2O and then partitioned with *n*-hexane, EtOAc, and *n*-butanol, successively. The *n*-hexane soluble fraction (26.3 g) was fractionated by vacuum liquid chromatography on silica gel 60 using a gradient *n*-hexane and EtOAc to give nine fractions (A–I). Fraction C (2.68 g) was chromatographed on a column of silica gel, eluted with a gradient of *n*-hexane–EtOAc (10:0–1:1), to give nine subfractions (C01–C09). Subfraction C05 was chromatographed on a column of silica gel, eluted with CH_2Cl_2:$CHCl_3$ (9.75:0.25), to give four subfractions (C05A-C05D). Subfraction C05B was separated on preparative TLC, eluted with *n*-hexane–EtOAc (9:1), to give compound **1** (5.3 mg). Fraction D (1.65 g) was chromatographed on a column of silica gel, eluted with a gradient of *n*-hexane–EtOAc (10:1–1:10), to give six subfractions (D01–D06). Subfraction D04 was recrystallized in EtOAc, to give compound **2** (27.6 mg). Subfraction D05 was chromatographed on a column of silica gel, eluted with a gradient of *n*-hexane–EtOAc (10:1–1:10) to afford five subfractions (D05A-D05E). Subfraction D05D was chromatographed on a column of silica gel, eluted with a gradient of $CHCl_3$–EtOAc (10:1–1:10) to give compound **3** (4.9 mg). The EtOAc soluble fraction (12.1 g) was fractionated by column chromatography on silica gel using a gradient *n*-hexane-EtOAc to give eight fractions (J–Q). Fraction K (927.6 mg) was chromatographed on a column of silica gel, eluted with *n*-hexane–EtOAc (10:1–1:1), to give four subfractions (K01–K04). Subfraction K02 was separated on preparative TLC silica gel GF_{254}, eluted with *n*-hexane:EtOAc (7:3), to give compound **4** (40.3 mg).

Compound **4** (12.0 mg) was dissolved in anhydrous pyridine (1 mL) in a vial (4 mL), and CrO_3 (20.0 mg) was then added. After standing at room temperature overnight, the reaction mixture was separated through a small silica gel (1 g) column (0.5 x 4.2 cm), eluted with *n*-hexane:Me_2CO (4:1, 20 mL). The elution was evaporated to dryness under reduced pressure at 45 °C, to give the oxidation product of compound **5**, 20-hydroxy-dammar-24-en-3-on (Rf 0.55; 4.2 mg).

Dammar-24-en-3α-ol (1). White needle-like crystals, m.p. 152-153 °C; IR (KBr) ν_{max} 3345, 2937, 2870, 1464, 1379, 1056 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 600 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 1.

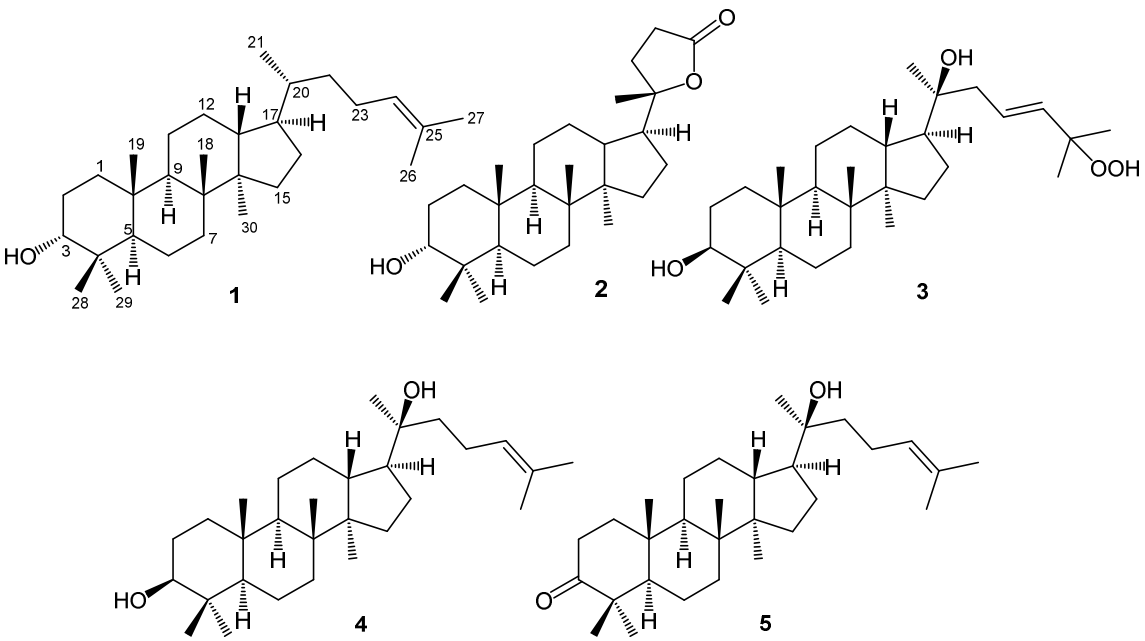

Fig 1. Structures of compounds **1**-**5**

3-*epi*-Cabraleahydroxy lactone (2). White amorphous powder, m.p. (decomposed); IR (KBr) ν_{max} 3477, 2942, 1715, 1471, 1387, 1075 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 600 MHz), ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 1; HR-TOFMS (positive ion mode) *m/z* 417.3105 $[M+H]^+$, (calcd. $C_{27}H_{44}O_3$, *m/z* 416.3290).

(*E*)-25-Hydroperoxydammar-23-en-3β,20-diol (3). Colorless oil; IR (KBr) ν_{max} 3436, 2945, 1651, 1456, 1074, 847 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 600 MHz), ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 1; HR-TOFMS (positive ion mode) *m/z* 477.3951 $[M+H]^+$, (calcd. $C_{30}H_{52}O_4$, *m/z* 476.3866).

Dammar-24-en-3β,20-diol (4). White amorphous powder, m.p. (decomposed); IR (KBr) ν_{max} 3369, 2939, 1639, 1458, 1109 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 600 MHz), ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 2; HR-TOFMS (positive ion mode) *m/z* 445.0527 $[M+H]^+$, (calcd. $C_{30}H_{52}O_2$, *m/z* 444.3967).

20-Hydroxy-dammar-24-en-3-on (5). White amorphous powder, m.p. (decomposed); ^{1}H-NMR ($CDCl_3$, 600 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 2.

Determination of Cytotoxic Activity

The P388 murine leukemia cells were seeded into 96-well plates at an initial cell density of approximately 3 x 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 tetra zolium 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 microplate 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, versus the tested concentration of compounds (µg/mL). The IC_{50} value is the concentration required for 50% growth inhibition. Each assay and analysis was run in triplicate and averaged [26-28].

RESULT AND DISCUSSION

Compound **1** was obtained as white needle-like crystal. The molecular formula of compound **1** was $C_{30}H_{52}O$ based on the analysis of NMR and thus required five degrees of unsaturation, originating from one pair of C *sp*^{2} and the remaining tetracyclic triterpenoids. The IR spectra showed absorption peaks at 3345 cm^{-1} (OH), 2937 and 2870 cm^{-1} (C-H *sp*^{3}), 1464 cm^{-1} (C=C), 1379 cm^{-1} (*gem*-dimethyl groups), and 1056 cm^{-1} (C-O). The ^{1}H-NMR ($CDCl_3$ 600 MHz) spectrum showed the presence of seven tertiary methyl groups, resonating at δ_H 0.95 (H-18), 0.85 (H-19), 1.62 (H-26), 1.56 (H-27), 0.96 (H-28), 0.79 (H-29), and 0.88 (H-30) and one secondary methyl at δ_H 1.10 (d, *J* = 6.5 Hz, H-21). There was one olefinic methine group, resonating at δ_H 5.09 (1H, t, *J* = 7 Hz, H-24) and

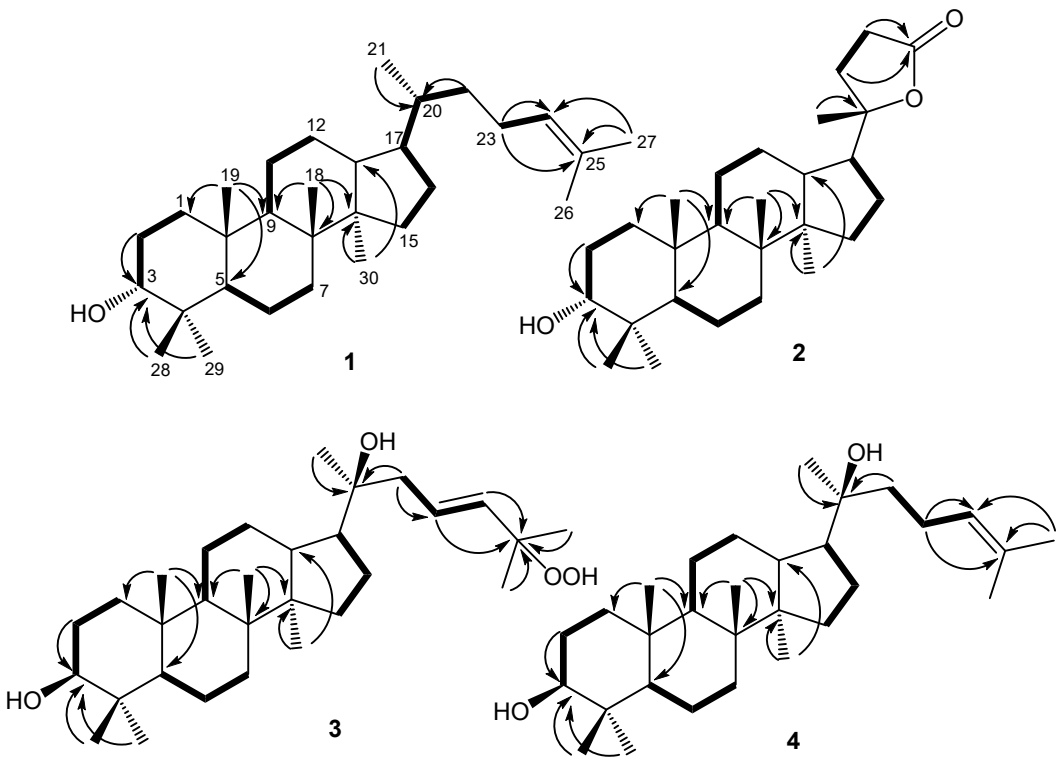

Fig 2. Selected HMBC and ^{1}H-^{1}H COSY correlations for **1**-**4**

one oxymethine resonating at δ_H 3.64 (1H, d, *J* = 2.5 Hz, H-3) which indicates that the hydroxy group was attached in C-3. The proton pairing was also confirmed with the ^{1}H-^{1}H COSY spectrum (Fig. 2). The ^{13}C-NMR ($CDCl_3$ 150 MHz) and DEPT 135° spectra showed the presence of eight methyl groups, exhibiting the characteristics of triterpenoid compounds [21-22], one olefinic methine at δ_C 125.3 (C-24), one olefinic quaternary carbon at δ_C 130.5 (C-25), and an oxymethine group at δ_C 75.0 (C-3). The HMBC cross-peaks (Fig. 2) from CH_3-28 (δ_H 0.96), CH_3-29 (δ_H 0.79), and the methylene protons at H-2 (δ_H 1.47) to the oxymethine carbon at C-3 (δ_C 75.0) indicated the presence of a hydroxy group at C-3. The correlation which was arising from CH_3-26 (δ_H 1.62) and CH_3-27 (δ_H 1.56) to C-25 (δ_C 130.5) and C-24 (δ_C 124.3) indicated that position of a double bond at C-24/C-25. The conformation of C-3 was assigned as α based on coupling constant of H-3 (*J* = 2.6 Hz) and by comparing to those of reference [29]. These functionalities accounted for one of five total degrees of unsaturation, and the remaining four degrees of unsaturation were consistent with the triterpenoid skeleton. A comparison of the NMR data of **1** with dammar-24-en-3α-ol [30] revealed that the structures of the two compounds were very similar; consequently, compound **1** was identified as dammar-24-en-3α-ol.

Compound **2** was obtained as a white amorphous powder. Its molecular composition $C_{27}H_{44}O_3$, was established from the HR-ESI-TOFMS spectrum (*m/z* 417.3105, $[M+H]^+$) together with NMR data (Table 1). The IR spectra showed absorption peaks at 3477 cm^{-1} (OH), 2942 cm^{-1} (C-H *sp*^{3}), 1715 cm^{-1} (C=O), 1471 and 1379 cm^{-1} (*gem*-dimethyl groups), and 1075 cm^{-1} (C-O). The ^{1}H-NMR ($CDCl_3$ 600 MHz) spectrum showed the presence of six tertiary methyl groups, resonating at δ_H 0.92 (CH_3-18), 0.82 (CH_3-19), 1.33 (CH_3-21), 0.91 (CH_3-28), 0.81 (CH_3-29), and 0.87 (CH_3-30) and one oxymethine group, resonating at δ_H 3.37 (1H, s, H-3) which was indicated the presence of dammarane-type triterpenoid skeleton [30-31]. The proton pairing was also confirmed with the ^{1}H-^{1}H COSY spectrum (Fig. 2). The ^{13}C-NMR ($CDCl_3$ 150 MHz) spectra showed 27 carbons and classified by DEPT 135° experiment as six methyl groups, exhibiting the characteristics of trisnor-triterpenoid compounds [31], one carbonyl lactone at δ_C 176.9 (C-24), an oxymethine group at δ_C 75.0 (C-3), and an oxygenated quaternary carbon at δ_C 90.3 (C-20). The HMBC cross-peaks (Fig. 2) from CH_3-28 (δ_H 0.91), CH_3-29 (δ_H 0.81), and the methylene protons at H-2 (δ_H 1.40) to the oxymethine carbon at C-3 (δ_C 76.3) indicated the presence of a hydroxy group at C-3. The correlation which was arising from H-22 (δ_H 1.47) and H-23 (δ_H 2.52) to C-24 (δ_C 176.9) and C-20 (δ_C 90.3) indicated that position of

Table 1. NMR Data (600 MHz for ^{1}H and 150 MHz for ^{13}C, in $CDCl_3$) for **1**-**5**

No.	1	2		3		4		5	
	^{13}C-NMR δc (mult.)	^{1}H-NMR δ_H (Int., mult, J=Hz)	^{13}C-NMR δc (mult.)	^{1}H-NMR δ_H (Int., mult, J=Hz)	^{13}C-NMR δc (mult.)	^{1}H-NMR δ_H (Int., mult, J=Hz)	^{13}C-NMR δc (mult.)	^{1}H-NMR δ_H (Int., mult, J=Hz)	^{1}H-NMR δ_H (Int., mult, J=Hz)
1	33.7 (t)	1.33 (2H, m)	35.2 (t)	1.17, 1.50 (each 1H, m)	39.1 (t)	1.68 (2H, dd, 3.6, 13.2)	33.7 (t)	1.37, 1.40 (each 1H, d, 1.2)	1.44, 1.93 (each 1H, m)
2	24.6 (t)	1.47 (2H, m)	33.7 (t)	1.40 (2H, dd, 2.4, 9.6)	24.9 (t)	1.44, 1.71 (each 1H, m)	24.9 (t)	1.43 (2H, m)	1.96, 2.48 (each 1H, m)
3	75.0 (d)	3.64 (1H, d, 2.6)	76.3 (d)	3.37 (1H, s)	79.1 (d)	3.19 (1H, dd, 4.8, 11.4)	76.4 (d)	3.37 (1H, t, 4.5)	-
4	37.5 (s)	-	37.3 (s)	-	39.0 (s)	-	37.7 (s)	-	-
5	49.2 (d)	1.36 (1H, dd, 2.4, 11.4)	49.4 (d)	1.95 (1H, m)	55.9 (d)	0.71 (1H, dd, 2.4, 9.6)	49.6 (d)	1.23 (1H, m)	1.39 (1H, t, 5.3)
6	18.1 (t)	1.40 (2H, m)	18.3 (t)	1.37 (2H, m)	18.3 (t)	1.40, 1.53 (each 1H, m)	18.3 (t)	1.38 (2H, m)	1.46 (2H, m)
7	35.2 (t)	1.23 (2H, m)	26.9 (t)	1.71 (2H, m)	35.3 (t)	1.25 (2H, m)	35.2 (t)	1.24, 1.55 (each 1H, m)	1.33 (2H, m)
8	40.6 (s)	-	40.6 (s)	-	40.4 (s)	-	40.7 (s)	-	-
9	49.5 (d)	1.71 (1H, t, 4.8)	50.4 (d)	1.41 (1H, dd, 2.4, 13.2)	50.7 (d)	1.29 (1H, m)	50.4 (d)	1.42 (1H, m)	1.41 (1H, t, 5.3)
10	37.2 (d)	-	37.7 (s)	-	37.2 (s)	-	37.3 (s)	-	-
11	21.3 (t)	1.51 (2H, m)	25.4 (t)	1.20 (2H, m)	21.6 (t)	1.22, 1.48 (each 1H, m)	21.4 (t)	1.52 (2H, m)	1.54 (2H, m)
12	25.7 (t)	1.09 (2H, m)	21.3 (t)	1.49, 1.91 (each 1H, m)	27.5 (t)	1.59 (2H, m)	25.4 (t)	1.53 (1H, m) 1.91 (1H, tt, 2.4, 13.1)	1.91 (2H, m)
13	42.0 (d)	1.71 (1H, m)	43.2 (d)	1.53 (1H, m)	42.5 (d)	1.63 (1H, m)	42.3 (d)	1.58 (1H, m)	1.69 (1H, m)
14	50.3 (s)	-	50.3 (s)	-	50.4 (s)	-	50.5 (s)	-	-
15	31.1 (t)	1.00 (2H, m)	31.2 (t)	1.90, 1.10 (each 1H, m)	31.2 (t)	1.07 (1H, dd, 1.8, 8.4) 1.45 (1H, m)	31.2 (t)	1.04 (1H, dd, 7.2, 11.4) 1.45 (1H, m)	1.61, 2.01 (each 1H, m)
16	27.7 (t)	1.13 (2H, m)	25.1 (t)	1.52 (2H, m)	27.6 (t)	1.21, 1.82 (each 1H, m)	27.6 (t)	1.77 (2H, m)	1.51, 1.98 (each 1H, m)
17	50.6 (d)	1.46 (1H, m)	49.5 (d)	1.23 (1H, m)	50.3 (d)	1.72 (1H, dd, 3.6, 6.6)	49.8 (d)	1.69 (1H, m)	1.71 (1H, m)
18	15.8 (q)	0.95 (3H, s)	15.6 (q)	0.92 (3H, s)	15.6 (q)	0.94 (3H, s)	15.6 (q)	0.93 (3H, s)	0.94 (3H, s)
19	15.1 (q)	0.85 (3H, s)	16.1 (q)	0.82 (3H, s)	16.5 (q)	0.83 (3H, s)	16.1 (q)	0.82 (3H, s)	0.83 (3H, s)
20	38.7 (d)	1.16 (1H, m)	90.3 (s)	-	75.2 (s)	-	75.5 (s)	-	-
21	25.1 (q)	1.10 (3H, d, 6.5)	25.4 (q)	1.33 (3H, s)	25.8 (q)	1.11 (3H, s)	25.5 (q)	1.13 (3H, s)	1.15 (3H, s)
22	41.2 (t)	1.42 (2H, m)	31.3 (t)	1.47, 2.01 (each 1H, m)	43.4 (t)	2.22 (2H, dd, 7.8, 11.4)	40.6 (t)	1.44 (2H, m)	1.45 (2H, m)
23	22.5 (t)	1.24 (2H, m)	29.3 (t)	2.52 (1H, d, 10) 2.62 (1H, d, 9.9)	127.4 (d)	5.76 (1H, dd, 7.8, 16.2)	22.6 (t)	2.02 (2H, q, 7.8)	2.01 (2H, q, 7.8)
24	125.3 (d)	5.09 (1H, t, 7.0)	176.9 (s)	-	137.4 (d)	5.60 (1H, dd, 4.8, 16.2)	124.8 (d)	5.10 (1H, t, 5.4)	5.13 (1H, t, 5.4)
25	130.5 (s)	-			82.2 (s)	-	131.7 (s)	-	-
26	25.2 (q)	1.62 (3H, s)			24.2 (q)	1.34 (3H, s)	25.9 (q)	1.66 (3H, s)	1.65 (3H, s)
27	16.9 (q)	1.56 (3H, s)			24.5 (q)	1.33 (3H, s)	17.8 (q)	1.59 (3H, s)	1.50 (3H, s)
28	28.3 (q)	0.96 (3H, s)	28.4 (q)	0.91 (3H, s)	28.1 (q)	0.96 (3H, s)	28.4 (q)	0.91 (3H, s)	0.92 (3H, s)
29	21.8 (q)	0.79 (3H, s)	22.2 (q)	0.81 (3H, s)	15.4 (q)	0.76 (3H, s)	22.2 (q)	0.81 (3H, s)	0.83 (3H, s)
30	16.2 (q)	0.88 (3H, s)	16.4 (q)	0.87 (3H, s)	16.3 (q)	0.85 (3H, s)	16.6 (q)	0.86 (3H, s)	0.86 (3H, s)

lactone in C-20/C-24. The conformation of C-3 was assigned as α based on coupling constant of H-3 (J = 0) [22,31]. These functionalities accounted for one of six total degrees of unsaturation, and the remaining five degrees of unsaturation were consistent with the triterpenoid skeleton with lactone ring at the side chain. A comparison of the NMR data of **2** with cabraleahydroxy lactone [22] revealed that the structures of the two compounds were very similar, consequently, compound **2** was identified as a 3-*epi*-cabraleahydroxy lactone.

Compound **3** was obtained as a colorless oil. Its molecular composition $C_{30}H_{52}O_4$, was established from the HR-ESI-TOFMS spectrum (*m/z* 477.3951, $[M+H]^+$) together with NMR data (Table 1). The IR spectra showed absorption peaks at 3436 cm^{-1} (OH), 2945 cm^{-1} (C-H sp^3), 1651 cm^{-1} (C=C), 1456 cm^{-1} (*gem*-dimethyl groups), 1076 cm^{-1} (C-O), and 847 cm^{-1} (O-O). The ^{1}H-NMR ($CDCl_3$ 600 MHz) spectrum showed the presence of eight tertiary methyl groups, resonating at δ_H 0.94 (CH_3-18), 0.83 (CH_3-19), 1.11 (CH_3-21), 1.34 (CH_3-26), 1.33 (CH_3-27), 0.96 (CH_3-28), 0.76 (CH_3-29), and 0.85 (CH_3-30), one oxymethine group, resonating at δ_H 3.19 (1H, dd, J = 4.8, 11.4 Hz, H-3), and two methine sp^2 at δ_H 5.76 (1H, dd, J = 7.8, 16.2 Hz) and 5.60 (1H, dd, J = 4.8, 16.2 Hz, H-24), which was indicated the presence of dammarane-type triterpenoid skeleton. The proton pairing was also confirmed with the ^{1}H-^{1}H COSY spectrum (Fig. 2). The ^{13}C-NMR ($CDCl_3$ 150 MHz) spectra showed 30 carbons and classified by DEPT 135° experiment as eight methyl groups, an oxymethine group at δ_C 79.1 (C-3), two oxygenated quaternary carbons at δ_C 75.2 (C-20) and 82.2 (C-24), and two methine sp^2 at δ_C 127.4 (C-23) and 137.4 (C-24). One oxygenated quaternary carbon at δ_C 82.2 (C-24) was more deshielded, indicate that hydroperoxy group attaches at C-24 [26]. The HMBC cross-peaks (Fig. 2) from H-28 (δ_H 0.96), H-29 (δ_H 0.76), and the methylene protons at H-2 (δ_H 1.44) to the oxymethine carbon at C-3 (δ_C 79.1) indicated the presence of a hydroxy group at C-3. The correlation which was arising from H-23 (δ_H 5.76) and H-24 (δ_H 5.60) to C-22 (δ_C 43.4) and C-25 (δ_C 82.2) suggest the position of a double bond at C-23/C-24. The conformation of C-3 was assigned as β based on coupling constant of H-3 (J = 4.8, 11.4 Hz) [22]. These functionalities accounted for one of five total degrees of unsaturation, and the remaining four degrees of unsaturation were consistent with the triterpenoid skeleton. A comparison of the NMR data of **3** with (*E*)-25-hydroperoxydammar-23-en-3β,20-diol [22] revealed that the structures of the two compounds were very similar; consequently, compound **3** was identified as 3(*E*)-25-hydroperoxydammar-23-en-3β,20-diol.

Compound **4** was obtained as a colorless oil. Its molecular composition $C_{30}H_{52}O_2$, was established from the HR-ESI-TOFMS spectrum (*m/z* 445.0527, $[M+H]^+$) together with NMR data (Table 1). The IR spectra showed absorption peaks at 3369 cm^{-1} (OH), 2939 cm^{-1} (C-H sp^3), 1639 cm^{-1} (C=C), 1458 cm^{-1} (*gem*-dimethyl groups), and 1109 cm^{-1} (C-O). The ^{1}H-NMR ($CDCl_3$ 600 MHz) spectrum showed the presence of eight tertiary methyl groups, resonating at δ_H 0.93 (CH_3-18), 0.82 (CH_3-19), 1.13 (CH_3-21), 1.66 (CH_3-26), 1.59 (CH_3-27), 0.91 (CH_3-28), 0.81 (CH_3-29), and 0.86 (CH_3-30),

Table 2. Cytotoxic activity of compounds **1**–**5** against P-388 murine leukemia cells

Compounds	IC_{50} (µg/mL)
Dammar-24-en-3α-ol (**1**)	9.09 ± 0.10
3-*epi*-Cabraleahydroxy lactone (**2**)	68.53 ± 0.08
(*E*)-25-Hydroperoxydammar-23-en-3β,20-diol (**3**)	5.89 ± 0.08
Dammar-24-en-3β,20-diol (**4**)	22.40 ± 0.11
20-Hydroxy-dammar-24-en-3-on (**5**)	11.53 ± 0.08

one oxymethine group, resonating at δ_H 3.37 (1H, t, *J* = 4.5 Hz, H-3), and one methine sp^2 at δ_H 5.10 (1H, t, *J* = 5.4 Hz, H-24) which was indicated the presence of dammarane-type triterpenoid skeleton. The proton pairing was also confirmed with the ^1H-^1H COSY spectrum (Fig. 2). The ^{13}C-NMR ($CDCl_3$ 150 MHz) spectra showed 30 carbons and classified by DEPT 135° experiment as eight methyl groups, an oxymethine group at δ_C 76.4 (C-3), one oxygenated quaternary carbon at δ_C 75.5 (C-20), one methine sp^2 at δ_C 124.8 (C-24) and one quartenary sp^2 carbon at δ_C 131.7 (C-25). The HMBC cross-peaks (Fig. 2) from CH_3-28 (δ_H 0.91), CH_3-29 (δ_H 0.81), and the methylene protons at H-2 (δ_H 1.43) to the oxymethine carbon at C-3 (δ_C 76.4) indicated the presence of a hydroxy group at C-3. The correlation which was arising from CH_3-21 (δ_H 1.13) and CH_3-22 (δ_H 1.44) to C-20 (δ_C 75.5) confirm that another hydroxy group at C-20. The position of a double bond at C-24/C-25 evidenced by the correlation between CH_3-26 (δ_H 1.66), CH_3-27 (δ_H 1.59), and H-23 (δ_H 2.02) to C-24 (δ_C 124.8) and C-25 (δ_C 131.7). The conformation of C-3 was assigned as β based on coupling constant of H-3 (*J* = 4.5 Hz) [33-34]. These functionalities accounted for one of five total degrees of unsaturation, and the remaining four degrees of unsaturation were consistent with the triterpenoid skeleton. A comparison of the NMR data of **4** with dammar-24-en-3β,20-diol [30,35] revealed that the structures of the two compounds were very similar; consequently, compound **4** was identified as dammar-24-en-3β,20-diol.

Compound **5** was obtained as a colorless oil. Its molecular composition $C_{30}H_{50}O_2$, was established from the NMR data (Table 1). The ^1H-NMR ($CDCl_3$ 600 MHz) spectrum showed similar with **4**. The difference was no signal for oxymethin at δ_H 3.37 (1H, t, *J* = 4.5 Hz, H-3), replace by carbonyl ketone. Indicate that oxidation product of **4** has formed.

The cytotoxic activity of the isolated compounds **1**-**4** and semi-synthetic compound **5** was evaluated against the P-388 murine leukemia cells according to a method described [23-24,28]. Artonin E (IC_{50} 0.75 µg/mL) was used as positive control [27]. The results are shown in Table 2. Among all dammarane-type triterpenoid compounds, (*E*)-25-hydroperoxydammar-23-en-3β,20-diol (**3**), having a hydroperoxy group and straight side chain, showed the strongest activity among the dammarane-type triterpenoids tested, whereas 3-*epi*-cabraleahydroxy lactone (**2**) showed weak activity, indicated the releasing of three carbons and lactonization in side chain, significantly decreasing the cytotoxic activity. These results suggested that a hydroperoxy group in the side chain may be some important structural features for cytotoxic activity in dammarane-type triterpenoids.

CONCLUSION

Four dammarane-type triterpenoid compounds, dammar-24-en-3α-ol (**1**), 3-*epi*-cabraleahydroxy lactone (**2**), (*E*)-25-hydroperoxydammar-23-en-3β,20-diol (**3**), and dammar-24-en-3β,20-diol (**4**), were isolated from the methanolic extract of the stembark of *Aglaia argentea*. Compounds, **1**-**4**, along with a known synthetic analog, 20-hydroxy-dammar-24-en-3-on (**5**), were evaluated their cytotoxic activity against P-388 murine leukemia cells *in vitro*. Among the dammarane-type triterpenoids, compounds **1**, **3**, **4** and **5** having opened side chain showed the stronger activity, where's compound **2** with cyclic side chain showed weak or no activity. In addition, compound **3** showed the strongest activity, indicate that hydroperoxy group at side chain increase cytotoxic activity.

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