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Cytotoxicity and Structure Activity Relationship of Dammarane-Type Triterpenoids from the Bark of *Aglaia elliptica* against P-388 Murine Leukemia Cells

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Abstract – Six dammarane-type triterpenoids, dammar-24-en-3β-ol (**1**), 3β-epicabraleahydroxy lactone (**2**), (*E*)-25-hydroperoxydammar-23-en-3β,20-diol (**3**), dammar-24-en-3β,20-diol (**4**), 3β-acetyl-20*S*,24*S*-epoxy-25-hydroxy-dammarane (**5**), and 3β-epiocotillol (**6**) were isolated from the methanolic extract of the bark of *Aglaia elliptica*. The chemical structure were identified on the basis of spectroscopic evidence and by comparison with those spectra previously reported. Compounds **1** - **6** were isolated first time from this plant. Compounds **1** - **6**, along with a known synthetic analog, cabraleone (**7**) were evaluated their cytotoxic activity against P-388 murine leukimia cells *in vitro*. Among those compounds 3β-acetyl-20*S*,24*S*-epoxy-25-hydroxydammarane (**5**) showed strongest cytotoxic activity with IC_{50} value of 8.02 ± 0.06 μM.
Keywords – Dammarane-type Triterpenoids, *Aglaia elliptica*, Meliaceae, P-388 murine leukimia cell

Introduction

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.[1] In our continous search for cytotoxic constituents against P-388 murine leukemia cells from Indonesian *Aglaia* plants, we isolated and described two new cytotoxic dammarane-type triterpenoids, aglinone and aglinin E, from the bark of *A. Smithii*.[2] In the further screening for cytotoxic compounds from Indonesia *Aglaia* plants, we found that the *n*-hexane and ethyl acetate extract of *A. elliptica* exhibited a cytotoxic activity against P-388 murine leukemia cells with IC_{50} values of 67.70 and 32.69 μg/mL, respectively. *A. elliptica* is a higher plant and widely distributed in South East Asia.[3,4] The plant is used in Indonesian folk medicine for the treatment of fever, diarrhea, contused wound, coughs and skin diseases.[4] Previous phytochemical studies on *Aglaia* plants reported the presence of rocaglamide,[5,6,7] bisamides,[8,9] sesquiterpenoids,[10,11] diterpenoids,[12,13] dammarane-type triterpenoids,[14-16] cycloartane-type triterpenoids,[17,18] and apotirucallane triterpenoids.[19,20] Although secondary metabolites of other *Aglaia* species have been investigated previously, the chemical composition of *A. elliptica*is yet to be reported. The isolation and structure identification of dammarane-type triterpenoids from the bark of *A. elliptica* along with cytotoxic evaluation against P-388 murine leukemia cells are described herein.

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Experimental

General experimental procedures – The IR spectra were measured on a Perkin Elmer spectrum-100 FT-IR in KBr. Mass spectra were obtained with a Water Qtof HR-MS XEV^{otm} and Water TQD MS/MS mass spectrometers. NMR spectra were recorded with a JEOL ECZ A-600 spectrometer using tetra methyl silane (TMS) as an internal standard. Chromatographic separation were carried out on

silica gel 60 (Merck). PTLC glass plates were precoated with silica gel GF_{254} (Merck, 0.25 mm). TLC plates were precoated with silica gel GF_{254} (Merck, 0.25 mm), detection was achieved with 10% H_2SO_4 in ethanol, followed by heating.

Plant materials – The bark of *A. elliptica* 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-1288719) was deposited at the herbarium.

Extraction and isolation – The dried bark (3.4 kg) of *A. elliptica* was extracted with methanol (10 L) at room temperature for 3 days. After removal the solvent, the viscous concentrate of MeOH extract (220 g) was first suspended in H_2O and then partitioned with *n*-hexane and EtOAc, successively. The EtOAc soluble fraction (43.5 g) was fractionated by column chromatography on silica gel 60 using a gradient *n*-hexane-EtOAc to give eight fractions (A-H). Fraction C (320 mg) was chromatographed on a column of silica gel, eluted successively with a gradient of *n*-hexane-acetone (10:1-1:1) to give seven fractions (C01-07). Fraction C03 (67 mg) was chromatographed on a column of silica gel, eluted with *n*-hexane:acetone (1:1) to give **1** (15.6 mg). Fraction D (220 mg) was chromatographed on a column of silica gel, eluted successively with a gradient of *n*-hexane-EtOAc (10:1-7:3) to give ten fractions (D01-10). Fraction D05-09 were combined (104 mg) and was chromatographed on silica gel, eluted with *n*-hexane:EtOAc (3:2) to give **2** (12.4 mg). Fraction E (220 mg) was chromatographed on a column of silica gel, eluted successively with a gradient of *n*-hexane-acetone (10:1-1:1) to give six fractions (E01-06). Fraction E03-05 were combined (82 mg) and was chromatographed on silica gel, eluted with *n*-hexane:EtOAc (1:2) to give **3** (12.4 mg). Fraction F (230 mg) was chromatographed on a column of silica gel, eluted successively with a gradient of *n*-hexane-acetone (20:1-1:1) to give five fractions (F01-05). Fraction F04 (78 mg) was chromatographed on a column of silica gel, eluted with gradient of *n*-hexane-acetone (10:1-1:1) to give **4** (12.3 mg). Fraction G (310 mg) was chromatographed on a column of silica gel, eluted successively with a gradient of *n*-hexane-acetone (10:1-1:1) to give eight subfractions (G01-08). Subfraction G06 (64 mg) was chromatographed on a column of silica gel, eluted with gradient of *n*-hexane-acetone (10:1-1:1) to give **5** (12.5 mg). Subfraction G08 (76 mg) was chromatographed on a column of silica gel, eluted with gradient of *n*-hexane-acetone (10:1-1:1) to give **6** (18.5 mg).

Compound **6** (10.0 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 × 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 **7**, cabraleone (R_f 0.75; 5.5 mg).

Dammar-24-en-3β-ol (**1**) – white needle-like crystals; m.p. 158 - 161 °C; IR (KBr) ν_{max} cm^{-1}: 3445, 2937, 2870, 1464, 1379, 1056; ^{1}H-NMR ($CDCl_3$, 600 MHz): δ_H 1.23 (1H, m, H-1a), 1.33 (1H, m, H-1b), 1.42 (1H, m, H-2a), 1.47 (1H, m, H-2b), 3.64 (1H, d, *J* = 2.6 Hz, H-3), 1.36 (1H, dd, *J* = 2.4, 11.4 Hz, H-5), 1.32 (1H, m, H-6a), 1.40 (1H, m, H-6b), 1.20 (1H, m, H-7a), 1.23 (1H, m, H-7b), 1.71 (1H, t, *J* = 4.8 Hz, H-9), 1.26 (1H, m, H-11a), 1.51 (1H, m, H-11b), 1.09 (1H, m, H-12a), 1.19 (1H, m, H-12b), 1.71 (1H, m, H-13), 1.07 (1H, m, H-15a), 1.17 (1H, m, H-15b), 1.13 (1H, m, H-16a), 1.15 (1H, m, H-16b), 1.46 (1H, m, H-17), 0.95 (3H, s, CH_3-18), 0.85 (3H, s, CH_3-19), 1.16 (1H, m, H-20), 1.10 (3H, d, *J* = 6.5 Hz, H-21), 1.36 (1H, m, H-22a), 1.42 (1H, m, H-22b), 1.19 (1H, m, H-23a), 1.24 (1H, m, H-23b), 5.09 (1H, t, *J* = 7.0 Hz, H-24), 1.62 (3H, s, CH_3-26), 1.56 (3H, s, CH_3-27), 0.96 (3H, s, CH_3-28), 0.79 (3H, s, CH_3-29), 0.88 (3H, s, CH_3-30); ^{13}C-NMR ($CDCl_3$, 125 MHz): Table 1.

3β-epicabraleahydroxy lactone (**2**) – white powder; IR (KBr) ν_{max} cm^{-1}: 3477, 2942, 1715, 1471, 1387, 1075; ^{1}H-NMR ($CDCl_3$, 600 MHz): δ_H 1.17 (1H, m, H-1a), 1.50 (1H, m, H-1b), 1.38 (1H, m, H-2a), 1.40 (1H, dd, *J* = 2.4, 9.6 Hz, H-2b), 3.37 (1H, ddd, *J* = 2.4, 6.8, 9.6 Hz, H-3), 1.95 (1H, m, H-5), 1.32 (1H, m, H-6a), 1.37 (1H, m, H-6b), 1.58 (1H, m, H-7a), 1.71 (1H, m, H-7b), 1.41 (1H, dd, *J* = 2.4, 13.2 Hz, H-9), 1.20 (1H, m, H-11a), 1.24 (1H, m, H-11b), 1.49 (1H, m, H-12a), 1.91 (1H, m, H-12b), 1.53 (1H, m, H-13), 1.10 (1H, m, H-15a), 1.90 (1H, m, H-15b), 1.46 (1H, m, H-16a), 1.52 (1H, m, H-16b), 1.23 (1H, m, H-17), 0.92 (3H, s, CH_3-18), 0.82 (3H, s, CH_3-19), 1.33 (3H, s, CH_3-21), 1.47 (1H, m, H-22a), 2.01 (1H, m, H-22b), 2.52 (1H, d, *J* = 9.9 Hz, H-23a), 2.62 (1H, d, *J* = 9.9 Hz, H-23b), 0.91 (3H, s, CH_3-28), 0.81 (3H, s, CH_3-29), 0.87 (3H, s, CH_3-30); (^{13}C-NMR ($CDCl_3$, 150 MHz): Table 1; HR-TOFMS *m/z* 417.3105 $[M+H]^+$, calcd. for $C_{27}H_{44}O_3$ *m/z* 416.32900.

(*E*)-25-hydroperoxydammar-23-en-3β,20-diol (**3**) – White amorphous powder; IR (KBr) ν_{max} cm^{-1}: 3436, 2945, 1639, 1456, 1074, 847; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 1.68 (1H, dd, *J* = 3.6, 13.2 Hz, H-1a), 1.56 (1H, dd, *J* = 3.6, 9.4 Hz, H-1b), 1.44 (1H, m, H-2a), 1.71 (1H, m, H-2b), 3.19 (1H, dd, *J* = 4.8, 11.4 Hz, H-3), 0.71 (1H, dd, *J* = 2.4, 9.6 Hz, H-5), 1.40 (1H, m, H-6a), 1.53 (1H, m,

H-6b), 1.25 (1H, m, H-7a), 1.28 (1H, m, H-7b), 1.29 (1H, m, H-9), 1.22 (1H, m, H-11a), 1.48 (1H, m, H-11b), 1.59 (1H, m, H-12a), 1.76 (1H, m, H-12b), 1.63 (1H, m, H-13), 1.07 (1H, dd, J = 1.8, 8.4 Hz, H-15a), 1.21 (1H, m, H-16a), 1.82 (1H, m, H-16b), 1.72 (1H, dd, J = 3.6, 6.6 Hz, H-17), 0.94 (3H, s, CH_3-18), 0.83 (3H, s, CH_3-19), 1.11 (3H, s, CH_3-21), 2.22 (1H, dd, J = 7.8, 11.4 Hz, H-22a), 2.34 (1H, m, H-22b), 5.76 (1H, dd, J = 7.8, 16.2 Hz, H-23), 5.60 (1H, dd, J = 4.8, 16.2 Hz, H-24), 1.34 (3H, s, CH_3-26), 1.33 (3H, s, CH_3-27), 0.96 (3H, s, CH_3-28), 0.76 (3H, s, CH_3-29), 0.85 (3H, s, CH_3-30); ^{13}C-NMR ($CDCl_3$, 125 MHz): Table 1; HR-TOFMS*m/z* $[M+H]^+$ 477.3951, calcd. for $C_{30}H_{52}O_4$*m/z* 476.3866.

Dammar-24-en-3β,20-diol (**4**) – White amorphous powder; IR (KBr) ν_{max} cm^{-1}: 3369, 2939, 1639, 1458, 1109; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 1.37 (1H, d, J = 1.2 Hz, H-1a), 1.40 (1H, d, J = 1.2 Hz, H-1b), 1.43 (1H, m, H-2a), 1.45 (1H, m, H-2b), 3.37 (1H, t, J = 4.5 Hz, H-3), 1.23 (1H, m, H-5), 1.38 (1H, m, H-6a), 1.48 (1H, m, H-6b), 1.24 (1H, m, H-7a), 1.55 (1H, m, H-7b), 1.42 (1H, m, H-9), 1.52 (1H, m, H-11a), 1.56 (1H, m, H-11b), 1.53 (1H, m, H-12a), 1.91 (1H, m, H-12b), 1.58 (1H, m, H-13), 1.04 (1H, dd, J = 7.2, 11.4 Hz, H-15a), 1.45 (1H, m, H-15b), 1.77 (1H, m, H-16a), 1.82 (1H, m, H-16b), 1.69 (1H, m, H-17), 0.93 (3H, s, CH_3-18), 0.82 (3H, s, CH_3-19), 1.13 (3H, s, CH_3-21), 1.44 (1H, m, H-22a), 1.52 (1H, m, H-22b), 1.85 (1H, m, H-23a), 2.02 (1H, m, H-23b), 5.10 (1H, t, J = 5.4 Hz, H-24), 1.66 (3H, s, CH_3-26), 1.59 (3H, s, CH_3-27), 0.91 (3H, s, CH_3-28), 0.81 (3H, s, CH_3-29), 0.86 (3H, s, CH_3-30); ^{13}C-NMR ($CDCl_3$, 125 MHz): Table 1; HR-TOFMS *m/z* 445.0527 $[M+H]^+$, calcd. for $C_{30}H_{52}O_2$ *m/z* 444.3967.

3β-acetyl-20*S*,24*S*-epoxy-25-hydroxydammarane (**5**) – whitesolid; IR (KBr) ν_{max} cm^{-1}: 3200, 2949, 1705, 1457, 1380, 1080; ^{1}H-NMR (CD_3OD, 500 MHz): δ_H 1.40 (1H, m, H-1a), 1.44 (1H, m, H-1b), 1.61 (1H, m, H-2a), 1.65 (1H, m, H-2b), 4.61 (1H, t, J = 3.0 Hz, H-3), 1.42 (1H, dd, J = 3.0, 12.0 Hz, H-5), 1.38 (1H, m, H-6a), 1.42 (1H, m, H-6b), 1.65 (m, H-7a), 1.84 (1H, m, H-7b), 1.20 (1H, m, H-9), 1.52 (1H, m, H-11a), 1.58 (1H, m, H-11b), 1.78 (1H, m, H-12a), 1.84 (1H, m, H-12b), 1.63 (1H, m, H-13), 1.05 (1H, dd, J = 7.2, 10.8 Hz, H-15a), 1.48 (1H, m, H-15b), 1.87 (1H, m, H-16a), 1.92 (1H, m, H-16b), 1.86 (1H, m, H-17), 0.96 (3H, s, CH_3-18), 0.85 (3H, s, CH_3-19), 1.14 (3H, s, CH_3-21), 1.22 (1H, m, H-22a), 1.30 (1H, m, H-22b), 1.75 (1H, m, H-23a), 1.85 (1H, m, H-23b), 3.63 (1H, dd, J = 4.8, 10.2 Hz, H-24), 1.18 (3H, s, CH_3-26), 1.10 (3H, s, CH_3-27), 0.82 (3H, s, CH_3-28), 0.86 (3H, s, CH_3-29), 0.90 (3H, s, CH_3-30), 2.08 (3H, s, CH_3-2'); ^{13}C-NMR (CD_3OD, 125 MHz): Table 1; HR-TOFMS *m/z* 473.3645 $[M-H]^-$, calcd. for $C_{30}H_{50}O_4$ *m/z* 474.3709.

3β-epiocotillol (**6**) – white solid; IR (KBr) ν_{max} cm^{-1}: 3200, 2949, 1705, 1457, 1380, 1080; ^{1}H-NMR (CD_3OD, 500 MHz): δ_H 1.40 (1H, m, H-1a), 1.44 (1H, m, H-1b), 1.61 (1H, m, H-2a), 1.65 (1H, m, H-2b), 4.61 (1H, t, J = 3.0 Hz, H-3), 1.42 (1H, dd, J = 3.0, 12.0 Hz, H-5), 1.38 (1H, m, H-6a), 1.42 (1H, m, H-6b), 1.65 (m, H-7a), 1.84 (1H, m, H-7b), 1.20 (1H, m, H-9), 1.52 (1H, m, H-11a), 1.58 (1H, m, H-11b), 1.78 (1H, m, H-12a), 1.84 (1H, m, H-12b), 1.63 (1H, m, H-13), 1.05 (1H, dd, J = 7.2, 10.8 Hz, H-15a), 1.48 (1H, m, H-15b), 1.87 (1H, m, H-16a), 1.92 (1H, m, H-16b), 1.86 (1H, m, H-17), 0.96 (3H, s, CH_3-18), 0.85 (3H, s, CH_3-19), 1.14 (3H, s, CH_3-21), 1.22 (1H, m, H-22a), 1.30 (1H, m, H-22b), 1.75 (1H, m, H-23a), 1.85 (1H, m, H-23b), 3.63 (1H, dd, J = 4.8, 10.2 Hz, H-24), 1.18 (3H, s, CH_3-26), 1.10 (3H, s, CH_3-27), 0.82 (3H, s, CH_3-28), 0.86 (3H, s, CH_3-29), 0.90 (3H, s, CH_3-30), 2.08 (3H, s, CH_3-29); ^{13}C-NMR (CD_3OD, 125 MHz): Table 1; HR-TOFMS *m/z* 473.3645 $[M-H]^-$, calcd. for $C_{30}H_{50}O_4$*m/z* 474.3709.

Cabraleone (**7**) – Yellow amorphous powder; IR (KBr) ν_{max} cm^{-1}: 3379, 2935, 1755, 1457, 1111; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 1.88 (2H, m, CH_2-1), 2.47 (2H, m, CH_2-2), 1.21 (1H, m, H-5), 1.37 (2H, m, CH_2-6), 1.66 (2H, m, CH_2-7), 1.46 (1H, m, H-9), 1.55 (2H, m, CH_2-11), 1.73 (2H, m, CH_2-12), 1.65 (1H, m, H-13), 1.06 (2H, m, CH_2-15), 1.57 (2H, m, CH_2-16), 1.46 (1H, m, H-17), 0.93 (3H, s, CH_3-18), 1.00 (3H, s, CH_3-19), 1.14 (3H, s, CH_3-21), 1.30 (2H, m, CH_2-22), 1.90 (2H, m, CH_2-23), 3.64 (1H, dd, J = 5.2 and 9.7 Hz, H-24), 1.18 (3H, s, CH_3-26), 1.11 (3H, s, CH_3-27), 1.03 (3H, s, CH_3-28), 0,87 (3H, s, CH_3-29), 1.07 (3H, s, CH_3-30); ^{13}C-NMR ($CDCl_3$, 125 MHz): Table 1.

Cytotoxicity assay – The P-388 cells were seeded into 96-well plates at an initial cell density of approximately 3×10^4 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 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. 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.

Result and Discussion

The methanol extract of the stembark of *A. elliptica* was successively partitioned with *n*-hexane, EtOAc and *n*-BuOH. Repeated column chromatography using silica gel of the EtOAc soluble fractions led to the isolation of six dammarane-type compounds (Fig. 1). The structures of the isolated compounds were determined by spectroscopic methods including 1D, 2D NMR and ESI-TOFMS and TQD MS/MS. To the best our knowledge, compounds **1** -**6**, were isolated from *A. elliptica* for the first time, together with a synthetic analog **7**.

Compound **1** was obtained as a white needle-like crystals. 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 pairs 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 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[2], 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 crosspeaks (Fig. 2) from H-28 (δ_H 0.96), H-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. Correlation which was arrising from H-26 (δ_H 1.62) and H-27 (δ_H 1.56) to C-25 (δ_C 130.5) and C-24 (δ_C 124.3) indicate that position of double bond at C-24/C-25. The conformation of C-3 was assign as α based on coupling constant of H-3 (J = 2.6 Hz).[21] 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-3β-ol[22] revealed that the structures of the two compounds were very similar; consequently, compound **1** was identified as a

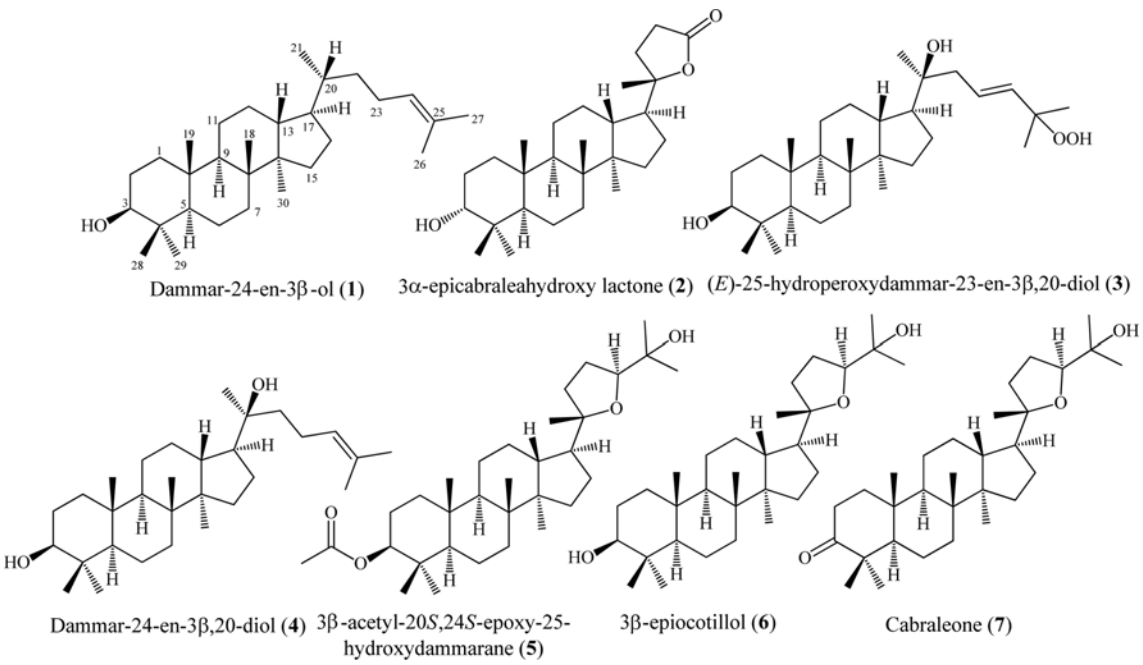

Fig. 1. The structures of **1** - **7** isolated from *A. elliptica*.

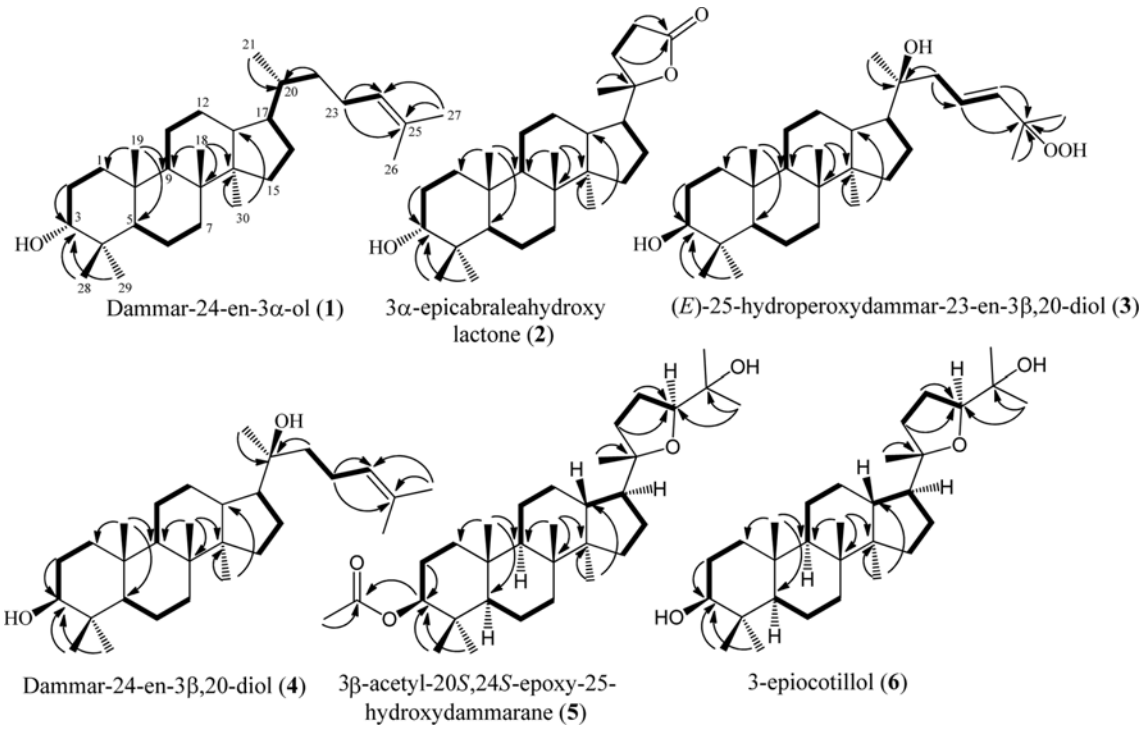

Fig. 2. Key HMBC (→) and COSY (▬) correlations of **1 - 6**.

dammar-24-en-3β-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 (H-18), 0.82 (H-19), 1.33 (H-21), 0.91 (H-28), 0.81 (H-29), and 0.87 (H-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. 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 tris nor-triterpenoid compounds[23], one carbonyl lactone at δ_C 176.9 (C-24), an oxymethine group at δ_C 75.0 (C-3), and an oxygenated quartenary carbon at δ_C 90.3 (C-20). The HMBC crosspeaks (Fig. 2) from H-28 (δ_H 0.91), H-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. Correlation which was arrising 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) indicate that position of lactone in C-20/C-24. The conformation of C-3 was assign as α based on coupling constant of H-3 ($J = 0$).[21] 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 side chain. A comparison of the NMR data of **2** with cabraleahydroxy lactone[23] revealed that the structures of the two compounds were very similar; consequently, compound **2** was identified as an 3α-*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 (H-18), 0.83 (H-19), 1.11 (H-21), 1.34 (H-26), 1.33 (H-27), 0.96 (H-28), 0.76 (H-29), and 0.85 (H-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

Table 1. ^{13}C-NMR data for compounds **1** - **7** (150 MHz in $CDCl_3$)

No.	**1** δc (mult.)	**2** δc (mult.)	**3** δc (mult.)	**4** δc (mult.)	**5** δc (mult.)	**6** δc (mult.)	**7** δc (mult.)
1	33.7 (t)	35.2 (t)	39.1 (t)	33.7 (t)	34.3 (t)	33.7 (t)	34.3 (t)
2	24.6 (t)	33.7 (t)	24.9 (t)	24.9 (t)	24.8 (t)	25.4 (t)	26.0 (t)
3	75.0 (d)	76.3 (d)	79.1 (d)	76.4 (d)	78.5 (d)	76.4 (d)	218.3 (s)
4	37.5 (s)	37.3 (s)	39.0 (s)	37.7 (s)	36.8 (s)	37.3 (s)	37.1 (s)
5	49.2 (d)	49.4 (d)	55.9 (d)	49.6 (d)	50.6 (d)	49.6 (d)	49.9 (d)
6	18.1 (t)	18.3 (t)	18.3 (t)	18.3 (t)	18.2 (t)	18.3 (t)	19.8 (t)
7	35.2 (t)	26.9 (t)	35.3 (t)	35.2 (t)	35.3 (t)	34.8 (t)	34.8 (t)
8	40.6 (s)	40.6 (s)	40.4 (s)	40.7 (s)	40.6 (s)	40.7 (s)	40.5 (s)
9	49.5 (d)	50.4 (d)	50.7 (d)	50.4 (d)	50.8 (d)	50.7 (d)	50.4 (d)
10	37.2 (d)	37.7 (s)	37.2 (s)	37.3 (s)	37.2 (s)	37.7 (s)	37.2 (s)
11	21.3 (t)	25.4 (t)	21.6 (t)	21.4 (t)	21.7 (t)	21.7 (t)	21.2 (t)
12	25.7 (t)	21.3 (t)	27.5 (t)	25.4 (t)	27.1 (t)	27.1 (t)	27.4 (t)
13	42.0 (d)	43.2 (d)	42.5 (d)	42.3 (d)	42.8 (d)	42.8 (d)	43.2 (d)
14	50.3 (s)	50.3 (s)	50.4 (s)	50.5 (s)	50.2 (s)	50.2 (s)	50.2 (s)
15	31.1 (t)	31.2 (t)	31.2 (t)	31.2 (t)	31.6 (t)	31.5 (t)	31.6 (t)
16	27.7 (t)	25.1 (t)	27.6 (t)	27.6 (t)	25.9 (t)	25.9 (t)	26.6 (t)
17	50.6 (d)	49.5 (d)	50.3 (d)	49.8 (d)	49.9 (d)	49.8 (d)	55.5 (d)
18	15.8 (q)	15.6 (q)	15.6 (q)	15.6 (q)	15.6 (q)	16.2 (q)	16.2 (q)
19	15.1 (q)	16.1 (q)	16.5 (q)	16.1 (q)	16.1 (q)	16.6 (q)	16.5 (q)
20	38.7 (d)	90.3 (s)	75.2 (s)	75.5 (s)	86.7 (s)	86.7 (s)	86.7 (s)
21	25.1 (q)	25.4 (q)	25.8 (q)	25.5 (q)	27.4 (q)	27.3 (q)	27.2 (q)
22	41.2 (t)	31.3 (t)	43.4 (t)	40.6 (t)	35.2 (t)	35.3 (t)	34.9 (t)
23	22.5 (t)	29.3 (t)	127.4 (d)	22.6 (t)	26.4 (t)	26.4 (t)	26.9 (t)
24	125.3 (d)	176.9 (s)	137.4 (d)	124.8 (d)	86.4 (d)	86.3 (d)	86.5 (d)
25	130.5 (s)		82.2 (s)	131.7 (s)	70.4 (s)	70.3 (s)	70.4 (s)
26	25.2 (q)		24.2 (q)	25.9 (q)	28.0 (q)	27.9 (q)	28.0 (q)
27	16.9 (q)		24.5 (q)	17.8 (q)	24.1 (q)	24.1 (q)	24.2 (q)
28	28.3 (q)	28.4 (q)	28.1 (q)	28.4 (q)	27.9 (q)	28.4 (q)	22.5 (q)
29	21.8 (q)	22.2 (q)	15.4 (q)	22.2 (q)	21.8 (q)	22.2 (q)	22.3 (q)
30	16.2 (q)	16.4 (q)	16.3 (q)	16.6 (q)	16.7 (q)	15.6 (q)	15.4 (q)
1'					171.1 (s)		
2'					21.5 (q)		

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 quartenary 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 quartenary carbon at δ_C 82.2 (C-24) was more deshielded, indicate that hydroperoxy group attach at C-24.[22] The HMBC crosspeaks (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. Correlation which was arrising 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) suggesting the position of double bond at C-23/C-24. The conformation of C-3 was assign as β based on coupling constant of H-3 (J = 4.8, 11.4 Hz).[21] 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 (H-18), 0.82 (H-19), 1.13 (H-21), 1.66 (H-26), 1.59 (H-27), 0.91 (H-28), 0.81 (H-29), and 0.86 (H-30), 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 ^{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 76.4 (C-3), one oxygenated quartenary 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 crosspeaks (Fig. 2) from H-28 (δ_H 0.91), H-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. Correlation which was arising from H-21 (δ_H 1.13) and H-22 (δ_H 1.44) to C-20 (δ_C 75.5) confirm that the another hydroxy group at C-20. The position of double bond at C-24/C-25 evidenced by correlation between H-26 (δ_H 1.66), H-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 assign as β based on coupling constant of H-3 (J = 4.5 Hz).[21] 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[24] 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 white solid. Its molecular composition $C_{32}H_{54}O_4$, was established from the HR-ESI-TOFMS spectrum (*m/z* 501.3770 $[M-H]^-$) together with NMR data (Table 1). The IR spectra showed absorption peaks at 3200 cm^{-1} (OH), 2949 cm^{-1} (C-H sp^3), 1705 cm^{-1} (C=O), 1457 and 1380 cm^{-1} (*gem*-dimethyl groups), and 1076 cm^{-1} (C-O). The ^{1}H-NMR ($CDCl_3$ 600 MHz) spectrum showed the presence of nine tertiary methyl groups, resonating at δ_H 0.96 (H-18), 0.85 (H-19), 1.14 (H-21), 1.18 (H-26), 1.10 (H-27), 0.82 (H-28), 0.86 (H-29), 0.90 (H-30), and 2.08 (H-2'), two oxymethine group, resonating at δ_H 4.61 (1H, t, J = 3 Hz, H-3) and 3.63 (1H, dd, J = 4.8, 10.2 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 nine methyl groups, two oxymethine group at δ_C 78.5 (C-3) and 86.4 (C-24), two oxygenated quartenary carbons at δ_C 86.7 (C-20) and 70.4 (C-25), and an ester group at δ_C 171.1 (C-1') correlated to acetyl group. The HMBC crosspeaks (Fig. 2) from H-28 (δ_H 0.82), H-29 (δ_H 0.86), and the methylene protons at H-2 (δ_H 1.61) to the oxymethine carbon at C-3 (δ_C 78.5) indicated the presence of a hydroxy group at C-3. The conformation of C-3 was assign as β based on coupling constant of H-3 (t, J = 3 Hz).[21] The position of acetyl group in C-3 was evidenced by correlation of H-3 (δ_H 4.61) and H-2' (δ_H 2.08) to C-1' (δ_C 171.1). Correlation which was arising from H-21 (δ_H 1.14) to C-20 (δ_C 86.7), 22 (δ_C 35.2), 17 (δ_C 49.9) and correlation from H-24 (δ_H 3.63) to C-20 (δ_C 86.7) suggest the position of epoxydation at C-20/C-24. The stereochemistry of C-24 assign to be *S* based on study of compounds with 20,24-epoxy structure. This observation showed that chemical shift of C-24 could be used to determine the sterochemistry, which is δ_C 83.2 for *R* conformer and δ_C 86.6 for*S* conformer.[14] A comparison of the NMR data of **5** with 3β-epiocotillol[14] revealed that the structures of the two compounds were different in acetyl group appearance; consequently, compound **5** was identified as 3β-acetyl-3-epiocotillol or 3β-acetyl-20*S*,24*S*-epoxy-25-hydroxydammarane.

Compound **6** was obtained as a white solid. Its molecular composition $C_{30}H_{52}O_3$, was established from the HR-ESI-TOFMS spectrum (*m/z* 461.3600 $[M+H]^+$) together with NMR data (Table 1). The IR spectra showed absorption peaks at 3457 cm^{-1} (OH), 2866 cm^{-1} (C-H sp^3), 1457 and 1380 cm^{-1} (*gem*-dimethyl groups), and 1055 cm^{-1} (C-O). The ^{1}H-NMR ($CDCl_3$ 600 MHz) spectrum showed the presence of eight tertiary methyl groups, with high similarity of chemical shift with compound **5**, the main difference is the absence af acetyl group resonating at δ_H 2.08 (H-2'), which was indicated that **6** is a deacetylated of **5**, which was a dammarane-type triterpenoid structure. The ^{13}C-NMR ($CDCl_3$ 150 MHz) spectra showed 30 carbons and classified by DEPT 135° experiment as eight methyl groups, two oxymethine groups, two oxygenated quartenary carbons. All of this ^{13}C NMR chemical shift is similar with **5**, the main difference is absence of ester group at δ_C 171.1 (C-1') and methyl group at δ_C 21.5 (C-2'), which were correlated to acetyl group. A comparison of the NMR data of **6** with 3-epiocotillol[14] revealed that the structures of the two compounds were very similar; consequently, compound **6** was identified as 3β-epiocotillol.

Compound **7** was obtained as a white amorphous powder. Its molecular composition $C_{30}H_{50}O_3$, was established from the NMR data (Table 1). The ^{1}H-NMR ($CDCl_3$ 500 MHz), ^{13}C-NMR ($CDCl_3$ 125 MHz), and DEPT 135° spectrum showed high similarity with 3-epiocotillol (compound **6**). The difference was no signal for oxymethin at δ_H 3.38 (1H, t, J = 3 Hz, H-3) and δ_C 76.4 (C-3), replace by carbonyl ketone (δ_C 218.3). Indicate that oxidation product of **6** has formed.

The cytotoxicity effects of the seven isolated compounds **1** - **6**, along with a synthetic product (**7**) against the P-388 murine leukemia cells were conducted according to

Table 2. Cytotoxicity activity of compounds **1** – **7** against P-388 murine leukemia cells

Compounds	IC_{50} (μM)
Dammar-24-en-3α-ol (**1**)	21.30 ± 0.06
3-epicabraleahydroxy lactone (**2**)	104.71 ± 0.05
(*E*)-25-hydroperoxydammar-23-en-3β,20-diol (**3**)	12.41 ± 0.04
Dammar-24-en-3β,20-diol (**4**)	50.44 ± 0.04
3α-acetyl-20*S*,24*S*-epoxy-25-hydroxydammarane (**5**)	8.20 ± 0.06
3-epiocotillol (**6**)	23.94 ± 0.04
Cabraleone (**7**)	32.86 ± 0.04
Artonin E*	0.68 ± 0.05

*Positive control

the method described in previous paper[2,20,25,26] and were used an Artonin E (IC_{50} 0.68 ± 0.05 μM) as a positive control.[27] The cytotoxicity activities of isolated compounds **1** - **7** are shown in Table 2. Among all dammarane-type triterpenoid compounds, 3α-acetyl-20*S*,24*S*-epoxy-25-hydroxydammarane (**5**), having acetyl group showed the strongest activity among the dammarane-type triterpenoids tested, whereas 3-*epi*-cabraleahydroxy lactone (**2**) showed weak activity, indicate the releasing of three carbons and lactonization in side chain, significanly decreasing the cytotoxic activity. (*E*)-25-hydroperoxydammar-23-en-3β, 20-diol (**3**), having a hydroperoxy group and straight side chain, also showed high cytotoxic activity. These results suggested that acetyl and hydroperoxy group in the side chain may be some important structural features for cytotoxic activity in dammarane-type triterpenoids.

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