



## 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-3 $\beta$ -ol (**1**), 3 $\beta$ -epicabraleahydroxy lactone (**2**), (*E*)-25-hydroperoxydammar-23-en-3 $\beta$ ,20-diol (**3**), dammar-24-en-3 $\beta$ ,20-diol (**4**), 3 $\beta$ -acetyl-20*S*,24*S*-epoxy-25-hydroxy-dammarane (**5**), and 3 $\beta$ -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 leukemia cells *in vitro*. Among those compounds 3 $\beta$ -acetyl-20*S*,24*S*-epoxy-25-hydroxydammarane (**5**) showed strongest cytotoxic activity with IC<sub>50</sub> value of 8.02  $\pm$  0.06  $\mu$ M.

**Keywords** – Dammarane-type Triterpenoids, *Aglaia elliptica*, Meliaceae, P-388 murine leukemia 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.<sup>1</sup> In our continuous 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*.<sup>2</sup> 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<sub>50</sub> values of 67.70 and 32.69  $\mu$ g/mL, respectively. *A. elliptica* is a higher plant and widely distributed in South East Asia.<sup>3,4</sup> The plant is used in Indonesian folk medicine for

the treatment of fever, diarrhea, contused wound, coughs and skin diseases.<sup>4</sup> Previous phytochemical studies on *Aglaia* plants reported the presence of rocaglamide,<sup>5,6,7</sup> bisamides,<sup>8,9</sup> sesquiterpenoids,<sup>10,11</sup> diterpenoids,<sup>12,13</sup> dammarane-type triterpenoids,<sup>14-16</sup> cycloartane-type triterpenoids,<sup>17,18</sup> and apotirucallane triterpenoids.<sup>19,20</sup> 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.

### 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 XEVO<sup>tm</sup> 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

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silica gel 60 (Merck). PTLC glass plates were precoated with silica gel GF<sub>254</sub> (Merck, 0.25 mm). TLC plates were precoated with silica gel GF<sub>254</sub> (Merck, 0.25 mm), detection was achieved with 10% H<sub>2</sub>SO<sub>4</sub> 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<sub>2</sub>O 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<sub>3</sub> (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<sub>2</sub>CO (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*<sub>f</sub> 0.75; 5.5 mg).

**Dammar-24-en-3β-ol (1)** – white needle-like crystals; m.p. 158 - 161 °C; IR (KBr)  $\nu_{\max}$  cm<sup>-1</sup>: 3445, 2937, 2870, 1464, 1379, 1056; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 600 MHz):  $\delta_{\text{H}}$  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<sub>3</sub>-18), 0.85 (3H, s, CH<sub>3</sub>-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<sub>3</sub>-26), 1.56 (3H, s, CH<sub>3</sub>-27), 0.96 (3H, s, CH<sub>3</sub>-28), 0.79 (3H, s, CH<sub>3</sub>-29), 0.88 (3H, s, CH<sub>3</sub>-30); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 125 MHz): Table 1.

**3β-epicabraleahydroxy lactone (2)** – white powder; IR (KBr)  $\nu_{\max}$  cm<sup>-1</sup>: 3477, 2942, 1715, 1471, 1387, 1075; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 600 MHz):  $\delta_{\text{H}}$  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<sub>3</sub>-18), 0.82 (3H, s, CH<sub>3</sub>-19), 1.33 (3H, s, CH<sub>3</sub>-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<sub>3</sub>-28), 0.81 (3H, s, CH<sub>3</sub>-29), 0.87 (3H, s, CH<sub>3</sub>-30); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 150 MHz): Table 1; HR-TOFMS *m/z* 417.3105 [M+H]<sup>+</sup>, calcd. for C<sub>27</sub>H<sub>44</sub>O<sub>3</sub> *m/z* 416.32900.

**(E)-25-hydroperoxydammar-23-en-3β,20-diol (3)** – White amorphous powder; IR (KBr)  $\nu_{\max}$  cm<sup>-1</sup>: 3436, 2945, 1639, 1456, 1074, 847; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 500 MHz):  $\delta_{\text{H}}$  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<sub>3</sub>-18), 0.83 (3H, s, CH<sub>3</sub>-19), 1.11 (3H, s, CH<sub>3</sub>-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<sub>3</sub>-26), 1.33 (3H, s, CH<sub>3</sub>-27), 0.96 (3H, s, CH<sub>3</sub>-28), 0.76 (3H, s, CH<sub>3</sub>-29), 0.85 (3H, s, CH<sub>3</sub>-30); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 125 MHz): Table 1; HR-TOFMS  $m/z$  [M+H]<sup>+</sup> 477.3951, calcd. for C<sub>30</sub>H<sub>52</sub>O<sub>4</sub>  $m/z$  476.3866.

**Damar-24-en-3β,20-diol (4)** – White amorphous powder; IR (KBr)  $\nu_{\max}$  cm<sup>-1</sup>: 3369, 2939, 1639, 1458, 1109; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 500 MHz):  $\delta_H$  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<sub>3</sub>-18), 0.82 (3H, s, CH<sub>3</sub>-19), 1.13 (3H, s, CH<sub>3</sub>-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<sub>3</sub>-26), 1.59 (3H, s, CH<sub>3</sub>-27), 0.91 (3H, s, CH<sub>3</sub>-28), 0.81 (3H, s, CH<sub>3</sub>-29), 0.86 (3H, s, CH<sub>3</sub>-30); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 125 MHz): Table 1; HR-TOFMS  $m/z$  445.0527 [M+H]<sup>+</sup>, calcd. for C<sub>30</sub>H<sub>52</sub>O<sub>2</sub>  $m/z$  444.3967.

**3β-acetyl-20S,24S-epoxy-25-hydroxydammarane (5)** – whitesolid; IR (KBr)  $\nu_{\max}$  cm<sup>-1</sup>: 3200, 2949, 1705, 1457, 1380, 1080; <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 500 MHz):  $\delta_H$  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<sub>3</sub>-18), 0.85 (3H, s, CH<sub>3</sub>-19), 1.14 (3H, s, CH<sub>3</sub>-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<sub>3</sub>-26), 1.10 (3H, s, CH<sub>3</sub>-27), 0.82 (3H, s, CH<sub>3</sub>-28), 0.86 (3H, s, CH<sub>3</sub>-29), 0.90 (3H, s, CH<sub>3</sub>-30), 2.08 (3H, s, CH<sub>3</sub>-2'); <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 125 MHz): Table 1; HR-TOFMS

$m/z$  473.3645 [M-H]<sup>-</sup>, calcd. for C<sub>30</sub>H<sub>50</sub>O<sub>4</sub>  $m/z$  474.3709.

**3β-epiocotillol (6)** – white solid; IR (KBr)  $\nu_{\max}$  cm<sup>-1</sup>: 3200, 2949, 1705, 1457, 1380, 1080; <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 500 MHz):  $\delta_H$  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<sub>3</sub>-18), 0.85 (3H, s, CH<sub>3</sub>-19), 1.14 (3H, s, CH<sub>3</sub>-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<sub>3</sub>-26), 1.10 (3H, s, CH<sub>3</sub>-27), 0.82 (3H, s, CH<sub>3</sub>-28), 0.86 (3H, s, CH<sub>3</sub>-29), 0.90 (3H, s, CH<sub>3</sub>-30), 2.08 (3H, s, CH<sub>3</sub>-29); <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 125 MHz): Table 1; HR-TOFMS  $m/z$  473.3645 [M-H]<sup>-</sup>, calcd. for C<sub>30</sub>H<sub>50</sub>O<sub>4</sub>  $m/z$  474.3709.

**Cabraleone (7)** – Yellow amorphous powder; IR (KBr)  $\nu_{\max}$  cm<sup>-1</sup>: 3379, 2935, 1755, 1457, 1111; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 500 MHz):  $\delta_H$  1.88 (2H, m, CH<sub>2</sub>-1), 2.47 (2H, m, CH<sub>2</sub>-2), 1.21 (1H, m, H-5), 1.37 (2H, m, CH<sub>2</sub>-6), 1.66 (2H, m, CH<sub>2</sub>-7), 1.46 (1H, m, H-9), 1.55 (2H, m, CH<sub>2</sub>-11), 1.73 (2H, m, CH<sub>2</sub>-12), 1.65 (1H, m, H-13), 1.06 (2H, m, CH<sub>2</sub>-15), 1.57 (2H, m, CH<sub>2</sub>-16), 1.46 (1H, m, H-17), 0.93 (3H, s, CH<sub>3</sub>-18), 1.00 (3H, s, CH<sub>3</sub>-19), 1.14 (3H, s, CH<sub>3</sub>-21), 1.30 (2H, m, CH<sub>2</sub>-22), 1.90 (2H, m, CH<sub>2</sub>-23), 3.64 (1H, dd,  $J=5.2$  and  $9.7$  Hz, H-24), 1.18 (3H, s, CH<sub>3</sub>-26), 1.11 (3H, s, CH<sub>3</sub>-27), 1.03 (3H, s, CH<sub>3</sub>-28), 0.87 (3H, s, CH<sub>3</sub>-29), 1.07 (3H, s, CH<sub>3</sub>-30); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 125 MHz): Table 1.

**Cytotoxicity assay** – The P-388 cells were seeded into 96-well plates at an initial cell density of approximately  $3 \times 10^4$  cells cm<sup>-3</sup>. 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<sub>50</sub> values were taken from the plotted graph of percentage

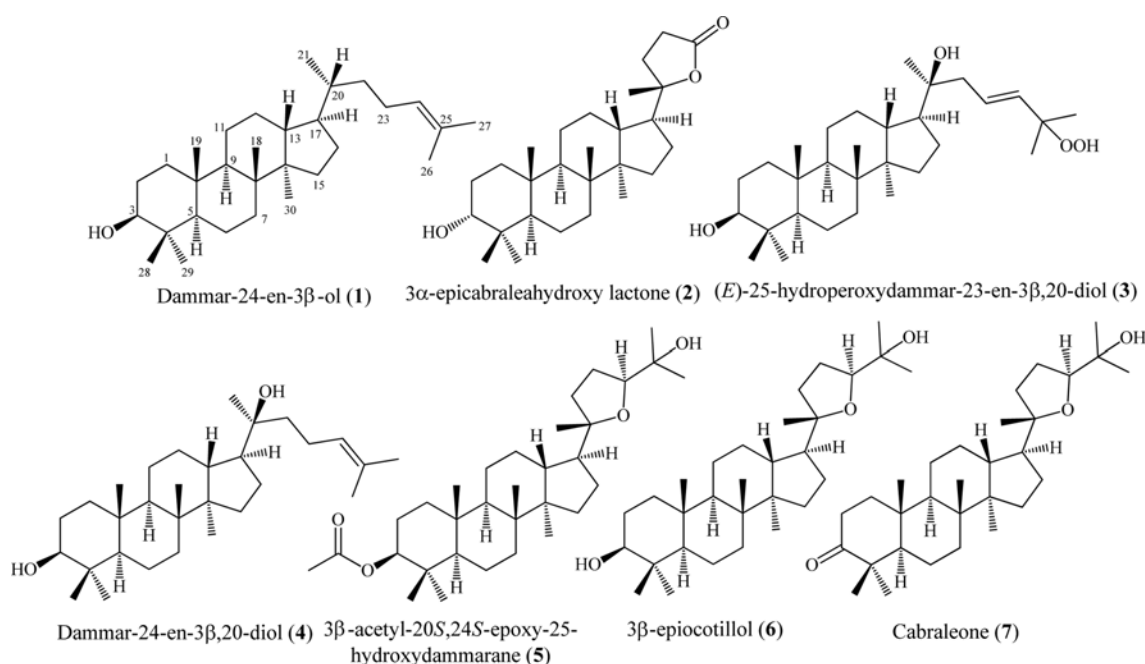
live cells compared to control (%), receiving only PBS and DMSO, versus the tested concentration of compounds ( $\mu\text{g/mL}$ ). The  $\text{IC}_{50}$  value is the concentration required for 50% growth inhibition. Each assay and analysis was run in triplicate and averaged.

## Result and Discussion

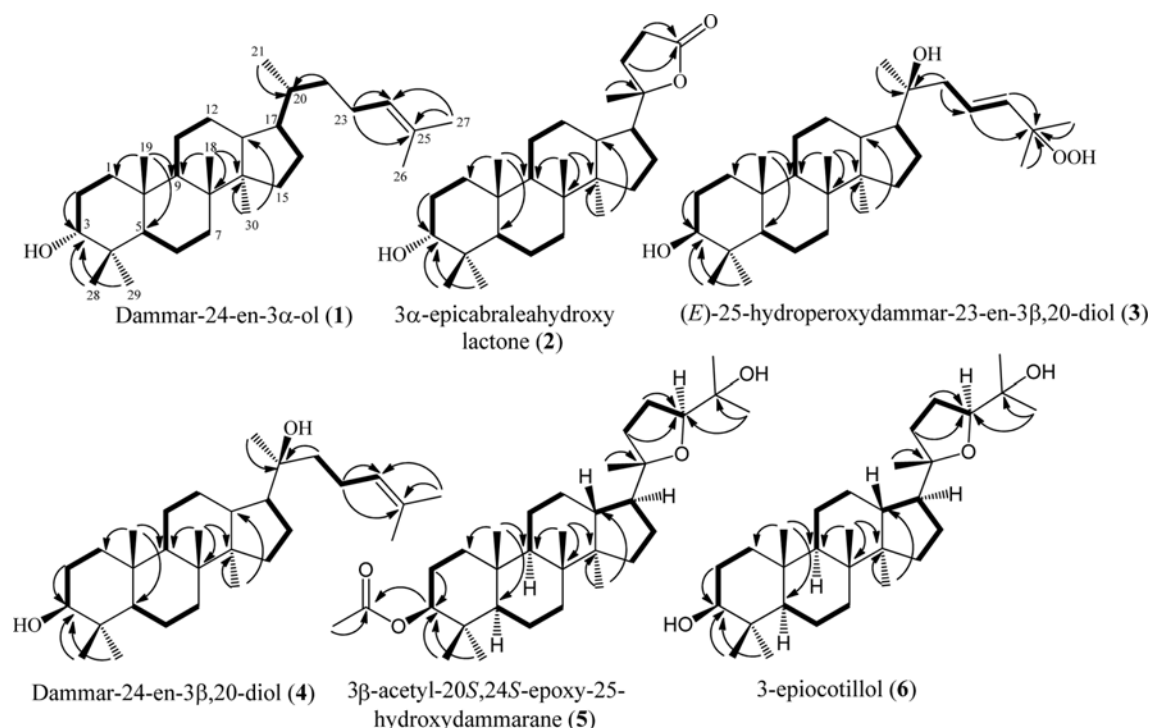
The methanol extract of the stem bark of *A. elliptica* was successively partitioned with *n*-hexane, EtOAc and *n*-BuOH. Repeated column chromatography using silica gel of the EtOAc soluble fractions led to the isolation of six dammarane-type compounds (Fig. 1). The structures of the isolated compounds were determined by spectroscopic methods including 1D, 2D NMR and ESI-TOFMS and TQD MS/MS. To the best of 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  $\text{C}_{30}\text{H}_{52}\text{O}$  based on the analysis of NMR and thus required five degrees of unsaturation, originating from one pair of  $\text{C } sp^2$  and the remaining tetracyclic triterpenoids. The IR spectra showed absorption peaks at  $3345\text{ cm}^{-1}$  (OH),  $2937$  and  $2870\text{ cm}^{-1}$  (C-H  $sp^3$ ),  $1464\text{ cm}^{-1}$  (C=C),  $1379\text{ cm}^{-1}$  (*gem*-dimethyl groups), and  $1056\text{ cm}^{-1}$  (C-O). The  $^1\text{H}$ -NMR ( $\text{CDCl}_3$  600 MHz) spectrum showed the presence of seven tertiary methyl groups, resonating at  $\delta_{\text{H}}$  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  $\delta_{\text{H}}$  1.10 (d,  $J=6.5\text{ Hz}$ , H-21). There was one olefinic methine group, resonating at  $\delta_{\text{H}}$  5.09 (1H, t,  $J=7\text{ Hz}$ , H-24) and one oxymethine resonating at  $\delta_{\text{H}}$  3.64 (1H, d,  $J=2.5\text{ Hz}$ , H-3) which indicates that the hydroxy group was attached in C-3. The proton pairing was also confirmed with the  $^1\text{H}$ - $^1\text{H}$  COSY spectrum (Fig. 2). The  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$  150 MHz) and DEPT  $135^\circ$  spectra showed the presence of eight methyl groups, exhibiting the characteristics of triterpenoid compounds<sup>2</sup>, one olefinic methine at  $\delta_{\text{C}}$  125.3 (C-24), one olefinic quaternary carbon at  $\delta_{\text{C}}$  130.5 (C-25), and an oxymethine group at  $\delta_{\text{C}}$  75.0 (C-3). The HMBC crosspeaks (Fig. 2) from H-28 ( $\delta_{\text{H}}$  0.96), H-29 ( $\delta_{\text{H}}$  0.79), and the methylene protons at H-2 ( $\delta_{\text{H}}$  1.47) to the oxymethine carbon at C-3 ( $\delta_{\text{C}}$  75.0) indicated the presence of a hydroxy group at C-3. Correlation which was arising from H-26 ( $\delta_{\text{H}}$  1.62) and H-27 ( $\delta_{\text{H}}$  1.56) to C-25 ( $\delta_{\text{C}}$  130.5) and C-24 ( $\delta_{\text{C}}$  124.3) indicate that position of double bond at C-24/C-25. The conformation of C-3 was assigned as  $\alpha$  based on coupling constant of H-3 ( $J=2.6\text{ Hz}$ ).<sup>21</sup> 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-3 $\beta$ -ol<sup>22</sup> 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 ( $\rightarrow$ ) and COSY ( $\text{—}$ ) correlations of **1** - **6**.

dammar-24-en-3 $\beta$ -ol.

Compound **2** was obtained as a white amorphous powder. Its molecular composition  $\text{C}_{27}\text{H}_{44}\text{O}_3$ , was established from the HR-ESI-TOFMS spectrum ( $m/z$  417.3105,  $[\text{M}+\text{H}]^+$ ) together with NMR data (Table 1). The IR spectra showed absorption peaks at  $3477\text{ cm}^{-1}$  (OH),  $2942\text{ cm}^{-1}$  (C-H  $sp^3$ ),  $1715\text{ cm}^{-1}$  (C=O),  $1471$  and  $1379\text{ cm}^{-1}$  (*gem*-dimethyl groups), and  $1075\text{ cm}^{-1}$  (C-O). The  $^1\text{H}$ -NMR ( $\text{CDCl}_3$  600 MHz) spectrum showed the presence of six tertiary methyl groups, resonating at  $\delta_{\text{H}}$  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  $\delta_{\text{H}}$  3.37 (1H, s, H-3) which indicated the presence of dammarane-type triterpenoid skeleton. The proton pairing was also confirmed with the  $^1\text{H}$ - $^1\text{H}$  COSY spectrum (Fig. 2). The  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$  150 MHz) spectra showed 27 carbons and classified by DEPT  $135^\circ$  experiment as six methyl groups, exhibiting the characteristics of tris nor-triterpenoid compounds<sup>23</sup>, one carbonyl lactone at  $\delta_{\text{C}}$  176.9 (C-24), an oxymethine group at  $\delta_{\text{C}}$  75.0 (C-3), and an oxygenated quaternary carbon at  $\delta_{\text{C}}$  90.3 (C-20). The HMBC crosspeaks (Fig. 2) from H-28 ( $\delta_{\text{H}}$  0.91), H-29 ( $\delta_{\text{H}}$  0.81), and the methylene protons at H-2 ( $\delta_{\text{H}}$  1.40) to the oxymethine carbon at C-3 ( $\delta_{\text{C}}$  76.3) indicated the presence of a hydroxy group at C-3. Correlation which was arising from H-22 ( $\delta_{\text{H}}$  1.47) and

H-23 ( $\delta_{\text{H}}$  2.52) to C-24 ( $\delta_{\text{C}}$  176.9) and C-20 ( $\delta_{\text{C}}$  90.3) indicate that position of lactone in C-20/C-24. The conformation of C-3 was assign as  $\alpha$  based on coupling constant of H-3 ( $J=0$ ).<sup>21</sup> 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<sup>23</sup> revealed that the structures of the two compounds were very similar; consequently, compound **2** was identified as an 3 $\alpha$ -*epi*-cabraleahydroxy lactone.

Compound **3** was obtained as a colorless oil. Its molecular composition  $\text{C}_{30}\text{H}_{52}\text{O}_4$ , was established from the HR-ESI-TOFMS spectrum ( $m/z$  477.3951,  $[\text{M}+\text{H}]^+$ ) together with NMR data (Table 1). The IR spectra showed absorption peaks at  $3436\text{ cm}^{-1}$  (OH),  $2945\text{ cm}^{-1}$  (C-H  $sp^3$ ),  $1651\text{ cm}^{-1}$  (C=C),  $1456\text{ cm}^{-1}$  (*gem*-dimethyl groups),  $1076\text{ cm}^{-1}$  (C-O), and  $847\text{ cm}^{-1}$  (O-O). The  $^1\text{H}$ -NMR ( $\text{CDCl}_3$  600 MHz) spectrum showed the presence of eight tertiary methyl groups, resonating at  $\delta_{\text{H}}$  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  $\delta_{\text{H}}$  3.19 (1H, dd,  $J=4.8, 11.4\text{ Hz}$ , H-3), and two methine  $sp^2$  at  $\delta_{\text{H}}$  5.76 (1H, dd,  $J=7.8, 16.2\text{ Hz}$ ) and 5.60 (1H, dd,  $J=4.8, 16.2\text{ Hz}$ , H-24), which was

**Table 1.**  $^{13}\text{C}$ -NMR data for compounds **1** - **7** (150 MHz in  $\text{CDCl}_3$ )

| No. | <b>1</b><br>$\delta_c$ (mult.) | <b>2</b><br>$\delta_c$ (mult.) | <b>3</b><br>$\delta_c$ (mult.) | <b>4</b><br>$\delta_c$ (mult.) | <b>5</b><br>$\delta_c$ (mult.) | <b>6</b><br>$\delta_c$ (mult.) | <b>7</b><br>$\delta_c$ (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  $^1\text{H}$ - $^1\text{H}$  COSY spectrum (Fig. 2). The  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ , 150 MHz) spectra showed 30 carbons and classified by DEPT 135° experiment as eight methyl groups, an oxymethine group at  $\delta_c$  79.1 (C-3), two oxygenated quaternary carbons at  $\delta_c$  75.2 (C-20) and 82.2 (C-24), and two methine  $sp^2$  at  $\delta_c$  127.4 (C-23) and 137.4 (C-24). One oxygenated quaternary carbon at  $\delta_c$  82.2 (C-24) was more deshielded, indicate that hydroperoxy group attach at C-24.<sup>22</sup> The HMBC crosspeaks (Fig. 2) from H-28 ( $\delta_H$  0.96), H-29 ( $\delta_H$  0.76), and the methylene protons at H-2 ( $\delta_H$  1.44) to the oxymethine carbon at C-3 ( $\delta_c$  79.1) indicated the presence of a hydroxy group at C-3. Correlation which was arising from H-23 ( $\delta_H$  5.76) and H-24 ( $\delta_H$  5.60) to C-22 ( $\delta_c$  43.4) and C-25 ( $\delta_c$  82.2) suggesting the position of double bond at C-23/C-24. The conformation of C-3 was assign as  $\beta$  based on coupling constant of H-3 ( $J=4.8$ , 11.4 Hz).<sup>21</sup> 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-3 $\beta$ ,20-diol<sup>22</sup> revealed that the structures of the two compounds were very similar; consequently, compound **3** was identified as 3(*E*)-25-hydroperoxydammar-23-en-3 $\beta$ ,20-diol.

Compound **4** was obtained as a colorless oil. Its molecular composition  $\text{C}_{30}\text{H}_{52}\text{O}_2$ , was established from the HR-ESI-TOFMS spectrum ( $m/z$  445.0527,  $[\text{M}+\text{H}]^+$ ) together with NMR data (Table 1). The IR spectra showed absorption peaks at  $3369\text{ cm}^{-1}$  (OH),  $2939\text{ cm}^{-1}$  (C-H  $sp^3$ ),  $1639\text{ cm}^{-1}$  (C=C),  $1458\text{ cm}^{-1}$  (*gem*-dimethyl groups), and  $1109\text{ cm}^{-1}$  (C-O). The  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ , 600 MHz) spectrum showed the presence of eight tertiary methyl groups, resonating at  $\delta_H$  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  $\delta_H$  3.37 (1H, t,  $J=4.5$  Hz, H-3), and one methine  $sp^2$  at  $\delta_H$  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  $^1\text{H}$ - $^1\text{H}$  COSY

spectrum (Fig. 2). The  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$  150 MHz) spectra showed 30 carbons and classified by DEPT 135° experiment as eight methyl groups, an oxymethine group at  $\delta_{\text{C}}$  76.4 (C-3), one oxygenated quaternary carbon at  $\delta_{\text{C}}$  75.5 (C-20), one methine  $sp^2$  at  $\delta_{\text{C}}$  124.8 (C-24) and one quaternary  $sp^2$  carbon at  $\delta_{\text{C}}$  131.7 (C-25). The HMBC crosspeaks (Fig. 2) from H-28 ( $\delta_{\text{H}}$  0.91), H-29 ( $\delta_{\text{H}}$  0.81), and the methylene protons at H-2 ( $\delta_{\text{H}}$  1.43) to the oxymethine carbon at C-3 ( $\delta_{\text{C}}$  76.4) indicated the presence of a hydroxy group at C-3. Correlation which was arising from H-21 ( $\delta_{\text{H}}$  1.13) and H-22 ( $\delta_{\text{H}}$  1.44) to C-20 ( $\delta_{\text{C}}$  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 ( $\delta_{\text{H}}$  1.66), H-27 ( $\delta_{\text{H}}$  1.59), and H-23 ( $\delta_{\text{H}}$  2.02) to C-24 ( $\delta_{\text{C}}$  124.8) and C-25 ( $\delta_{\text{C}}$  131.7). The conformation of C-3 was assign as  $\beta$  based on coupling constant of H-3 ( $J=4.5$  Hz).<sup>21</sup> 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-3 $\beta$ ,20-diol<sup>24</sup> revealed that the structures of the two compounds were very similar; consequently, compound **4** was identified as dammar-24-en-3 $\beta$ ,20-diol.

Compound **5** was obtained as a white solid. Its molecular composition  $\text{C}_{32}\text{H}_{54}\text{O}_4$ , was established from the HR-ESI-TOFMS spectrum ( $m/z$  501.3770  $[\text{M}-\text{H}]^-$ ) together with NMR data (Table 1). The IR spectra showed absorption peaks at  $3200\text{ cm}^{-1}$  (OH),  $2949\text{ cm}^{-1}$  (C-H  $sp^3$ ),  $1705\text{ cm}^{-1}$  (C=O),  $1457$  and  $1380\text{ cm}^{-1}$  (*gem*-dimethyl groups), and  $1076\text{ cm}^{-1}$  (C-O). The  $^1\text{H}$ -NMR ( $\text{CDCl}_3$  600 MHz) spectrum showed the presence of nine tertiary methyl groups, resonating at  $\delta_{\text{H}}$  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  $\delta_{\text{H}}$  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  $^1\text{H}$ - $^1\text{H}$  COSY spectrum (Fig. 2). The  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$  150 MHz) spectra showed 30 carbons and classified by DEPT 135° experiment as nine methyl groups, two oxymethine group at  $\delta_{\text{C}}$  78.5 (C-3) and 86.4 (C-24), two oxygenated quaternary carbons at  $\delta_{\text{C}}$  86.7 (C-20) and 70.4 (C-25), and an ester group at  $\delta_{\text{C}}$  171.1 (C-1') correlated to acetyl group. The HMBC crosspeaks (Fig. 2) from H-28 ( $\delta_{\text{H}}$  0.82), H-29 ( $\delta_{\text{H}}$  0.86), and the methylene protons at H-2 ( $\delta_{\text{H}}$  1.61) to the oxymethine carbon at C-3 ( $\delta_{\text{C}}$  78.5) indicated the presence of a hydroxy group at C-3. The conformation of C-3 was assign as  $\beta$  based on coupling constant of H-3 (t,  $J=3$

Hz).<sup>21</sup> The position of acetyl group in C-3 was evidenced by correlation of H-3 ( $\delta_{\text{H}}$  4.61) and H-2' ( $\delta_{\text{H}}$  2.08) to C-1' ( $\delta_{\text{C}}$  171.1). Correlation which was arising from H-21 ( $\delta_{\text{H}}$  1.14) to C-20 ( $\delta_{\text{C}}$  86.7), 22 ( $\delta_{\text{C}}$  35.2), 17 ( $\delta_{\text{C}}$  49.9) and correlation from H-24 ( $\delta_{\text{H}}$  3.63) to C-20 ( $\delta_{\text{C}}$  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 stereochemistry, which is  $\delta_{\text{C}}$  83.2 for *R* conformer and  $\delta_{\text{C}}$  86.6 for *S* conformer.<sup>14</sup> A comparison of the NMR data of **5** with 3 $\beta$ -epiocotillol<sup>14</sup> revealed that the structures of the two compounds were different in acetyl group appearance; consequently, compound **5** was identified as 3 $\beta$ -acetyl-3-epiocotillol or 3 $\beta$ -acetyl-20*S*,24*S*-epoxy-25-hydroxydammarane.

Compound **6** was obtained as a white solid. Its molecular composition  $\text{C}_{30}\text{H}_{52}\text{O}_3$ , was established from the HR-ESI-TOFMS spectrum ( $m/z$  461.3600  $[\text{M}+\text{H}]^+$ ) together with NMR data (Table 1). The IR spectra showed absorption peaks at  $3457\text{ cm}^{-1}$  (OH),  $2866\text{ cm}^{-1}$  (C-H  $sp^3$ ),  $1457$  and  $1380\text{ cm}^{-1}$  (*gem*-dimethyl groups), and  $1055\text{ cm}^{-1}$  (C-O). The  $^1\text{H}$ -NMR ( $\text{CDCl}_3$  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 of acetyl group resonating at  $\delta_{\text{H}}$  2.08 (H-2'), which was indicated that **6** is a deacetylated of **5**, which was a dammarane-type triterpenoid structure. The  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$  150 MHz) spectra showed 30 carbons and classified by DEPT 135° experiment as eight methyl groups, two oxymethine groups, two oxygenated quaternary carbons. All of this  $^{13}\text{C}$  NMR chemical shift is similar with **5**, the main difference is absence of ester group at  $\delta_{\text{C}}$  171.1 (C-1') and methyl group at  $\delta_{\text{C}}$  21.5 (C-2'), which were correlated to acetyl group. A comparison of the NMR data of **6** with 3-epiocotillol<sup>14</sup> revealed that the structures of the two compounds were very similar; consequently, compound **6** was identified as 3 $\beta$ -epiocotillol.

Compound **7** was obtained as a white amorphous powder. Its molecular composition  $\text{C}_{30}\text{H}_{50}\text{O}_3$ , was established from the NMR data (Table 1). The  $^1\text{H}$ -NMR ( $\text{CDCl}_3$  500 MHz),  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$  125 MHz), and DEPT 135° spectrum showed high similarity with 3-epiocotillol (compound **6**). The difference was no signal for oxymethine at  $\delta_{\text{H}}$  3.38 (1H, t,  $J=3$  Hz, H-3) and  $\delta_{\text{C}}$  76.4 (C-3), replace by carbonyl ketone ( $\delta_{\text{C}}$  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 <sub>50</sub> (μM) |
|--|-----------------------|
| Dammar-24-en-3α-ol ( <b>1</b> )  | 21.30 ± 0.06          |
| 3-epicabraleahydroxy lactone ( <b>2</b> )                                  | 104.71 ± 0.05         |
| ( <i>E</i> )-25-hydroperoxydammar-23-en-3β,20-diol ( <b>3</b> )            | 12.41 ± 0.04          |
| Dammar-24-en-3β,20-diol ( <b>4</b> )                                       | 50.44 ± 0.04          |
| 3α-acetyl-20 <i>S</i> ,24 <i>S</i> -epoxy-25-hydroxydammarane ( <b>5</b> ) | 8.20 ± 0.06           |
| 3-epicotillol ( <b>6</b> )   | 23.94 ± 0.04          |
| Cabraleone ( <b>7</b> )  | 32.86 ± 0.04          |
| Artonin E*   | 0.68 ± 0.05           |

\*Positive control

the method described in previous paper<sup>2,20,25,26</sup> and were used an Artonin E (IC<sub>50</sub> 0.68 ± 0.05 μM) as a positive control.<sup>27</sup> 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, significantly 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.

### Acknowledgments

This investigation was financially supported by Directorate General of Higher Education, Ministry of Research, Technology and Higher Education, Indonesia (Postgraduate Grant, 2015-2017, by US). We thank Mrs. Suzany Dwi Elita at Department of Chemistry, Faculty of Mathematics and Natural Sciences, Institute Technology Bandung, Indonesia for cytotoxicity bioassay.

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Received September 15, 2017

Revised October 2, 2017

Accepted October 3, 2017