Dammarane-Type Triterpenoid from the Stem Bark of Aglaia elliptica (Meliaceae) and Its Cytotoxic Activities

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Abstract: Two new dammarane-type triterpenoid fatty acid ester derivatives, 3β-oleate-20S-hydroxydammar-24-en (1) and 3β-oleate-20S,24S-epoxy-25-hydroxydammarane (2) with a known dammarane-type triterpenoid compound, such as 20S-hydroxydammar-24-en-3-on (3), were isolated from the stem bark of Aglaia elliptica (C.DC.) Blume. The chemical structures were determined by spectroscopic methods, including FTIR, NMR (one and two-dimensional), and HRESITOF-MS analysis, as well as chemical derivatization and comparison with previous literature. Furthermore, the synthetic analog resulting from transesterification of 1 and 2 also obtained 3β,20S-dihydroxy-dammar-24-en (4) and 20S,24S-epoxy-3β,25-dihydroxydammarane (5), respectively. The cytotoxic effect of all isolated and synthetic analog compounds was evaluated using PrestoBlue reagent against MCF-7 breast cancer cell and B16-F10 melanoma cell lines. The 20S-hydroxydammar-24-en-3-on (3) showed the strongest activity against MCF-7 breast cancer and B16-F10 melanoma cell, indicating that the ketone group at C-3 in 3 plays an essential role in the cytotoxicity of dammarane-type triterpenoid. On the other hand, compounds 1 and 2 had very weak cytotoxic activity against the two cell lines, indicating the presence of fatty acid, significantly decreasing cytotoxic activity. This showed the significance of the discovery to investigate the essential structural feature in dammarane-type triterpenoid, specifically for the future development of anticancer drugs.

Keywords: dammarane-type triterpenoid fatty acid ester; Aglaia elliptica; cytotoxic activity; MCF-7 cell line; B16-F10 cell line

1. Introduction

The Aglaia is the largest genus of the Meliaceae family, consisting of 150 species mainly distributed in tropical and sub-tropical regions such as Asia, Northern Australia, and the Pacific. Furthermore, approximately 65 species grow in Indonesia [1,2]. This plant is used traditionally in the country to treat wounds, fever, and skin disease [3]. On the other hand, phytochemical research of Aglaia genus revealed a number of diterpenoids [4,5], triterpenoids [6,7], sesquiterpenoids [8,9], limonoids [10,11], steroids [12], flavaglines [13], bisamides [14], and lignans [15,16]. The biological activity of the extracts and secondary metabolites includes cytotoxic [17,18], insecticidal [19], anti-inflammatory [5], antifungal [20], and molluscicide [21].

Dammarane triterpenoid is one of the secondary metabolite groups commonly discovered in the Aglaia genus. Approximately 29 dammarane-type triterpenoids have been...
successively isolated from the Aglaia genus [1]. This type of compound showed various bioactivity, such as cytotoxic activity. Zhang et al. [22] isolated six new dammarane triterpenoid compounds that showed potential cytotoxicity against leukemia cancer cell (K562), hepatocellular carcinoma (SMMC-7721), breast (MCF-7), and oral epithelial cancer (KB). Novel dammarane-type triterpenoids isolated from A. eximia and A. smithii were cytotoxic against P388 murin leukemia cells [6,23]. Oktaviani et al. [24] isolated three dammarane-type triterpenoids and showed cytotoxicity against cervical (HeLa) and human prostate cancer cells (DU145). Therefore, they can be used as an anticancer agent in the future.

Aglaia elliptica is a member of the Aglaia genus, which is widely grown in Indonesia, specifically on Kalimantan Island [12]. Previous research revealed its potency in producing compounds with cytotoxic activity against cancer cell lines [12,25,26]. There is a need for further exploration, since only 9 dammarane-type triterpenoids have been isolated from this species.

The isolation and structure elucidation of two new dammarane-type triterpenoid fatty acid esters, namely 3β-oleate-205-hydroxydammar-24-en (1) and 3β-oleate-205,24E-epoxy-25-hydroxydammarane (2), using a variety of chromatographic and spectroscopic techniques are described in this research. Furthermore, a known dammarane-type triterpenoid (3) and its synthetic analogs were reported through transesterification of 1 and 2 (4 and 5, respectively), as shown in Figure 1. The compounds were tested for their cytotoxic activity against MCF-7 breast cancer cells and B16-F10 melanoma cell lines. Therefore, this research briefly explains the structure-activity relationship of 1-5 against those cancer cell lines.

![Figure 1. Structure of compounds 1-5.](image-url)

2. Results and Discussions

2.1. Structural Elucidation of the Isolated Compounds

The concentrated methanolic extract of stem bark of A. elliptica was dissolved in water and extracted successively with n-hexane, ethyl acetate, and n-butanol. The n-hexane extract has rich triterpenoid content, as shown by the positive Liebermann–Burchard test, resulting in a dark purple color. Therefore, it was subjected to further separation and purification. The separation was conducted with vacuum liquid chromatography (VLC), followed by a combination of column chromatography on silica gel 60 to yield three cytotoxic dammarane-type triterpenoids 1-3, as presented in Figure 1. The structure elucidation of new dammarane-type triterpenoid fatty acid esters 1 and 2 were discussed based on spectroscopic evidence.
3β-oleate-20S-hydroxydammar-24-en (1) was isolated as a colorless oil. The HR-ESI-TOFMS spectra revealed the molecular composition of 1 as C₄₇H₈₁O₅ (m/z 709.6489 [M+H]+ calcd. for C₄₈H₈₄O₅, m/z 709.6493), which was established with the NMR data, as shown in Table 1. The IR spectrum indicates the presence of hydroxyl (3490 cm⁻¹), a carbonyl ester (1729 cm⁻¹), olefinic (1630 cm⁻¹), and gem-dimethyl groups (1375 cm⁻¹). Furthermore, the ¹³C NMR spectrum, with DEPT 135⁰ and assigned by HMQC spectra, revealed the presence of nine methyls, eight methines (including three sp² methines at δC 124.8, 128.9, and 131.8, and one oxymethine at δC 80.7), and seven quarternary carbons (including one carbonyl ester at δC 173.7, one olefinic carbon at δC 131.7, and one oxygenated quaternary carbon at δC 75.5). The overlapping signal at δC 29.2–29.6 indicated the presence of fatty acid substituent in 1. Three functionalities were observed from seven degrees of unsaturation, and the remaining four degrees accounted for the tetracyclic core of the dammarane-type triterpenoid. The ¹H-NMR spectrum showed eight tertiary methyls (δH 0.83, 0.85, 0.86, 0.94, 1.12, 1.61, 1.67, each 3H), one primary methyl (δH 0.85, 3H, J = 6.5 Hz), three olefinic proton at δH 5.10 (1H, t, J = 7.0 Hz), 5.35 (1H, dd, J = 3.5, 9.5 Hz), and 5.40 (1H, dd, J = 3.5, 9.5 Hz), as well as one oxymethine proton (δH 4.47, 1H, dd, J = 5.5, 10.5 Hz). A series of overlapping protons at δH 1.24 with high integral suggests the presence of fatty acid substituent. The comparison of NMR data of 1 with 3β,20S-dihydroxy-dammar-24-en was isolated from the same species by Hidayat et al. [25] showed high similarity. The primary difference was the presence of fatty acid substituent through ester linkage in 1. This comparison strengthens the presumption that compound 1 was a fatty acid ester derivative of 3β,20S-dihydroxy-dammar-24-en. On the other hand, the established structure was mainly determined by HMBC and ¹³C-¹H COSY experiments, as shown in Figure 2. The HMBC correlations of each tertiary methyl to the neighboring carbons confirmed the tetracyclic dammarane-type triterpenoid core.

The position of the double bond was assigned to be in C-24/C-25 from the correlations of CH₃-26 (δH 1.67) and CH₃-27 (δH 1.61) to C-24 (δC 124.8) and C-25 (δC 131.7) with a ¹H-¹H COSY correlation of H-22/H-23/H-24. Furthermore, the hydroxy group, bounded at C-20, was determined by the correlation of CH₃-21 (δH 1.12) to C-20 (δC 75.5), C-17 (δC 49.4), and C-22 (δC 40.6). The primary structural feature that determined the novelty of 1 was determined by the correlation of H-3 (δH 4.47), H-2' (δH 2.27), and H-3' (δH 1.59) to ester C-1' (δC 173.7), which supported the formation of ester linkage in 1 at C-3, with the confirmation of fatty acid substituent position. Furthermore, the type of fatty acid substituent at dammarane-type triterpenoid was determined by the mass spectrum of the transesterification products of 1 [27]. The mass spectrum of the transesterification products of 1 resulted in the identification of methyl oleate [M+H]+ m/z 297.2778 (calcd. for C₁₉H₃₇O₂⁺, m/z 297.2794) and the dammarane-type triterpenoid core moiety corresponds to 3β,20S-dihydroxy-dammar-24-en (4) based on HR-ESI-TOFMS and NMR data comparison [25]. The HMBC correlation was observed at H-9' (δH 5.40) to C-8' (δC 32.0) and C-11' (δC 32.1), whereas the ¹H-¹H COSY cross peak at H-8'/H-9'/H-10'/H-11' was supported by the presence of oleic acid, which has a double bond at C-9'/C-10'. On the other hand, the relative configuration of 1 was deduced by a NOESY experiment, as shown in Figure 3. The key NOESY correlations observed at H-3/H-5 indicated that the ester group at C-3 was β-oriented. The NOESY cross peak, which was also studied between CH₃-30/H-17, indicated that the side chain of 1 attached at C-17 was α-oriented. Absolute configuration of the hydroxyl group at C-20 was established to be S based on comparing chemical shifts with previously reported analogs [25,28]. Therefore, the structure of 1 as a new dammarane triterpenoid fatty acid ester derivative, 3β-oleate-20S-hydroxydammar-24-en, was elucidated.
| No. | $^{13}$C NMR $\delta_c$ (Mult.) | $^{1}$H NMR $\delta_{H}$ (Integral, Mult., $J = \text{Hz}$) | $^{13}$C NMR $\delta_c$ (Mult.) | $^{1}$H NMR $\delta_{H}$ (Integral, Mult., $J = \text{Hz}$) |
|-----|-------------------------------|-----------------------------------------------------|-------------------------------|-----------------------------------------------------|
| 1   | 32.7 (t)                      | 1.95 (2H, m)                                       | 33.0 (t)                      | 2.21 (2H, m)                                        |
| 2   | 24.9 (t)                      | 1.48 (2H, m)                                       | 23.8 (t)                      | 1.68 (2H, m)                                        |
| 3   | 80.7 (d)                      | 4.47 (1H, dd, 5.5, 10.5)                           | 80.6 (d)                      | 4.45 (1H, dd, 5.0, 11.0)                            |
| 4   | 38.0 (s)                      | -                                                  | 37.1 (s)                      | -                                                  |
| 5   | 56.0 (d)                      | 0.82 (1H, m)                                       | 56.0 (d)                      | 0.82 (1H, m)                                        |
| 6   | 18.2 (t)                      | 1.41 (2H, m)                                       | 18.2 (t)                      | 1.51, 1.38 (each 1H, m)                             |
| 7   | 35.2 (t)                      | 1.22, 1.54 (each 1H, m)                            | 34.8 (t)                      | 1.65 (1H, m)                                        |
| 8   | 40.4 (s)                      | -                                                  | 40.5 (s)                      | -                                                  |
| 9   | 50.6 (d)                      | 1.32 (1H, m)                                       | 50.8 (d)                      | 1.32 (1H, m)                                        |
| 10  | 37.1 (s)                      | -                                                  | 38.0 (s)                      | -                                                  |
| 11  | 21.6 (t)                      | 1.48 (2H, m)                                       | 21.1 (t)                      | 1.51 (2H, m)                                        |
| 12  | 25.1 (t)                      | 1.43 (2H, m)                                       | 27.0 (t)                      | 1.76 (2H, m)                                        |
| 13  | 42.3 (d)                      | 1.59 (1H, m)                                       | 42.9 (d)                      | 1.61 (1H, m)                                        |
| 14  | 50.4 (s)                      | -                                                  | 50.1 (s)                      | -                                                  |
| 15  | 31.2 (t)                      | 1.05 (2H, m)                                       | 31.5 (t)                      | 1.04 (2H, m)                                        |
| 16  | 27.6 (t)                      | 1.78 (2H, m)                                       | 25.9 (t)                      | 1.25 (2H, m)                                        |
| 17  | 49.9 (d)                      | 1.71 (1H, m)                                       | 49.9 (d)                      | 1.85 (1H, m)                                        |
| 18  | 15.6 (q)                      | 0.94 (3H, s)                                       | 16.4 (q)                      | 0.85 (3H, s)                                        |
| 19  | 16.3 (q)                      | 0.85 (3H, s)                                       | 16.6 (q)                      | 0.87 (3H, s)                                        |
| 20  | 75.5 (s)                      | -                                                  | 86.6 (s)                      | -                                                  |
| 21  | 25.5 (q)                      | 1.12 (3H, s)                                       | 27.2 (q)                      | 1.12 (3H, s)                                        |
| 22  | 40.6 (t)                      | 1.45 (2H, m)                                       | 35.3 (t)                      | 1.26 (2H, m)                                        |
| 23  | 22.7 (t)                      | 1.28 (2H, m)                                       | 26.4 (t)                      | 1.74 (2H, m)                                        |
| 24  | 124.8 (d)                     | 5.10 (1H, t, 7.0)                                  | 86.3 (d)                      | 3.62 (1H, dd, 5.5, 10.0)                            |
| 25  | 131.7 (s)                     | -                                                  | 70.3 (s)                      | -                                                  |
| 26  | 25.8 (q)                      | 1.67 (3H, s)                                       | 27.9 (q)                      | 1.17 (3H, s)                                        |
| 27  | 17.8 (q)                      | 1.61 (3H, s)                                       | 24.1 (q)                      | 1.09 (3H, s)                                        |
| 28  | 28.1 (q)                      | 0.83 (3H, s)                                       | 28.1 (q)                      | 0.82 (3H, s)                                        |
| 29  | 16.5 (q)                      | 0.85 (3H, s)                                       | 17.5 (q)                      | 1.16 (3H, s)                                        |
| 30  | 16.6 (q)                      | 0.86 (3H, s)                                       | 15.6 (q)                      | 0.95 (3H, s)                                        |
| 1'  | 173.7 (s)                     | -                                                  | 173.6 (s)                     | -                                                  |
| 2'  | 34.2 (t)                      | 2.27 (2H, t, 7.5)                                  | 34.2 (t)                      | 2.27 (2H, t, 7.5)                                  |
| 3'  | 23.8 (t)                      | 1.59 (2H, m)                                       | 23.8 (t)                      | 1.68 (2H, m)                                        |
| 4'  | 29.2 (t)                      | 1.24 (2H, m)                                       | 29.2 (t)                      | 1.24 (2H, m)                                        |
| 5'  | 29.6 (t)                      | 1.24 (2H, m)                                       | 29.6 (t)                      | 1.24 (2H, m)                                        |
| 6'  | 29.4 (t)                      | 1.24 (2H, m)                                       | 29.3 (t)                      | 1.24 (2H, m)                                        |
| 7'  | 29.7 (t)                      | 1.24 (2H, m)                                       | 29.7 (t)                      | 1.24 (2H, m)                                        |
| 8'  | 32.0 (t)                      | 1.99 (2H, m)                                       | 32.0 (t)                      | 2.00 (2H, m)                                        |
| 9'  | 128.9 (d)                     | 5.40 (1H, dd, 3.5, 9.5)                             | 128.9 (d)                     | 5.40 (1H, dd, 3.5, 10.0)                            |
| 10' | 131.8 (d)                     | 5.35 (1H, dd, 3.5, 9.5)                             | 131.7 (d)                     | 5.35 (1H, dd, 3.5, 10.0)                            |
| 11' | 32.1 (t)                      | 1.99 (2H, m)                                       | 32.1 (t)                      | 2.00 (2H, m)                                        |
| 12' | 29.4 (t)                      | 1.24 (2H, m)                                       | 29.3 (t)                      | 1.24 (2H, m)                                        |
| 13' | 29.6 (t)                      | 1.24 (2H, m)                                       | 29.6 (t)                      | 1.24 (2H, m)                                        |
| 14' | 29.2 (t)                      | 1.24 (2H, m)                                       | 29.2 (t)                      | 1.24 (2H, m)                                        |
| 15' | 29.4 (t)                      | 1.24 (2H, m)                                       | 29.3 (t)                      | 1.24 (2H, m)                                        |
| 16' | 29.7 (t)                      | 1.24 (2H, m)                                       | 29.7 (t)                      | 1.24 (2H, m)                                        |
| 17' | 22.6 (t)                      | 1.22 (2H, m)                                       | 22.7 (t)                      | 1.22 (2H, m)                                        |
| 18' | 14.2 (q)                      | 0.85 (3H, t, 6.5)                                  | 14.2 (q)                      | 0.85 (3H, t, 6.5)                                  |
Figure 2. Selected HMBC and $^1$H-$^1$H COSY correlations for 1 and 2.

Figure 3. Selected NOESY correlations for 1 and 2.

3β-oleate-20S,24S-epoxy-25-hydroxydammarane (2) was obtained as a colorless oil. The molecular composition of C$_{48}$H$_{84}$O$_4$ was established from the HR-ESI-TOFMS spectrum $m/z$ 725.6444 [M+H]$^+$ (calcd. for C$_{48}$H$_{85}$O$_4$+$^+$, $m/z$ 725.6442) with NMR data, as presented in Table 1. The IR spectrum showed the presence of hydroxyl, a carbonyl ester, olefinic, ether, and gem-dimethyl groups at 3511 cm$^{-1}$, 1731 cm$^{-1}$, 1647 cm$^{-1}$, 1173 cm$^{-1}$, and 1376 cm$^{-1}$, respectively. Furthermore, the $^{13}$C NMR spectrum detailed by DEPT 135° and HMQC spectra showed the resonances of nine methyls, eight methines (including two sp$^2$ methines at $\delta_C$ 128.9 and 131.7, and two oxymethines at $\delta_C$ 80.6 and 86.3), and seven quaternary carbons (including one carbonyl ester at $\delta_C$ 173.6 and two oxygenated quaternary carbon at $\delta_C$ 70.3 and 86.6). The overlapping carbon signal at approximately $\delta_C$ 29.2–29.6 indicated the presence of fatty acid substituent in 2. There are seven degrees of unsaturation, where two observed functionalities and the remaining five degrees were consistent for a tetracyclic dammarane-type triterpenoid core with an epoxide ring in the side chain. The $^1$H-NMR spectrum showed eight tertiary methyls ($\delta_H$ 0.82, 0.85, 0.87, 0.95, 1.09, 1.12, 1.16, 1.17, each 3H), one primary methyl at $\delta_H$ 0.85 (3H, t, $J$ = 6.5 Hz), two olefinic proton at $\delta_H$ 5.35 and 5.40 (each 1H, dd, $J$ = 3.5, 9.5 Hz), and two oxymethylene proton at $\delta_H$ 3.62 (1H, dd, $J$ = 5.5 Hz, 10.5 Hz) and 4.45 (1H, dd, $J$ = 5.0, 11.0 Hz). A series of overlapping protons at $\delta_H$ 1.24 with high integral predicts the fatty acid substituent in 2. Additionally, a detailed NMR data comparison of 2 with the 20S,24S-epoxy-3β,25-dihydroxydammarane isolated from the same species [25] showed that the structures of the two compounds are closely related. The main difference was the presence of a fatty acid ester substituent in 2, and the exact structure of 2 was determined by $^1$H-$^1$H COSY and HMBC spectra, as shown in Figure 2.

The tetracyclic dammarane-type triterpenoid core was established based on the correlation of tertiary methyl to the neighboring carbons. The hydroxyl group attached at C-25 was confirmed by the correlation of CH$_3$-26 ($\delta_H$ 1.17) and CH$_2$-27 ($\delta_H$ 1.09) to C-24 ($\delta_C$ 86.3) and C-25 ($\delta_C$ 70.3). Furthermore, the formation of tetrahydrofuran or epoxide rings through C-20/C-24 was mainly determined based on the correlation of CH$_3$-21 ($\delta_H$ 1.12) to C-20 ($\delta_C$ 86.6), C-17 ($\delta_C$ 49.9), and C-22 ($\delta_C$ 35.3), as well as H-23 ($\delta_H$ 1.74) to C-24 ($\delta_C$ 86.3). The $^1$H-$^1$H COSY cross peak of H-22/H-23/H-24 also supported the epoxide ring at
C-20/C-24. The ester group between triterpenoid moiety and fatty acid substituent was determined by the correlation of H-3 (δH 4.45), H-2' (δH 2.27), and H-3' (δH 1.68) to ester C-1' (δC 173.6) through ester linkage in 2 at C-3. Fatty acid substituent was determined using the same methods as compound 1. Moreover, the mass spectrum resulted in the identification of methyl oleate [M+H]+ m/z 297.2777 (calcd. for C19H32O2+, m/z 297.2794), and the dammarane-type triterpenoid core moiety corresponds to 20S,24S-epoxy-3β,25-dihydroxydammarane (5) based on the comparison of HR-ESI-TOFMS and NMR data [25]. The HMBC correlation was observed at H-9' (δH 5.40) to C-8' (δC 32.0) and C-11' (δC 32.1), whereas the 1H-1H COSY cross peak at H-8'/H-9'/H-10'/H-11' was supported by the presence of oleic acid which has a double bond at C-9'/C-10'. According to Figure 3, the relative configuration of 2 was deduced by a NOESY experiment, and the correlations observed at H-3/H-5 indicated that the ester group at C-3 was β-oriented. The NOESY cross peak, also observed between CH3-30/H-17, indicated that the side chain of 1 attached at C-17 was α-oriented. The absolute configuration of C-20 and C-24 was established to be the 20S and 24S based on the chemical shift of epoxy dammarane triterpenoid analogs [21,28]. Consequently, compound 2 was selected as 3β-oleate-20S,24S-epoxy-25-hydroxydammarane, a new dammarane-type triterpenoid fatty acid ester.

20S-hydroxydammar-24-en-3-on (3) was obtained as a white amorphous powder. 1H-NMR (CDCl3, 500 MHz) δH 1.86 (2H, H-1), 2.40 (2H, H-2), 1.32 (1H, H-5), 1.40 & 1.49 (each 1H, m, H-6), 1.26 & 1.56 (each 1H, m, H-7), 1.36 (1H, m, H-9), 1.25 & 1.45 (each 1H, m, H-11), 1.23 & 1.79 (each 1H, m, H-12), 1.69 (1H, m, H-13), 1.03 & 1.40 (each 1H, m, H-15), 1.44 & 1.69 (each 1H, m, H-16), 1.68 (1H, m, H-17), 0.93 (3H, s, H-18), 0.88 (3H, s, H-19), 1.08 (3H, s, H-21), 1.42 (2H, m, H-22), 1.99 (2H, m, H-23), 5.05 (1H, t, J = 5.0 Hz, H-24), 1.62 (3H, s, H-26), 1.56 (3H, s, H-27), 1.01 (3H, s, H-28), 0.97 (3H, s, H-29), 0.82 (3H, s, H-30). 13C-NMR (CDCl3, 125 MHz) δC 39.9 (C-1), 34.1 (C-2), 218.0 (C-3), 47.4 (C-4), 55.4 (C-5), 19.7 (C-6), 34.6 (C-7), 40.3 (C-8), 50.0 (C-9), 36.8 (C-10), 22.0 (C-11), 27.5 (C-12), 42.4 (C-13), 50.3 (C-14), 31.2 (C-15), 24.8 (C-16), 49.8 (C-17), 15.2 (C-18), 16.0 (C-19), 75.4 (C-20), 25.5 (C-21), 22.6 (C-22), 124.8 (C-24), 131.6 (C-25), 25.7 (C-26), 17.7 (C-27), 26.7 (C-28), 21.0 (C-29), 16.4 (C-30). HR-TOFMS m/z 443.3882 [M+H]+ (calcd. for C30H51O2+, m/z 443.3884). Compound 3 had a comparable HR-TOFMS result and chemical shift to 20S-hydroxydammar-24-en-3-on [29]. Therefore, it was identified as 20S-hydroxydammar-24-en-3-on, isolated for the first time from this species.

3β,20S-dihydroxy-dammar-24-en-4 (4) was obtained as a colorless crystal. 1H-NMR (CDCl3, 500 MHz) δH 1.37 & 1.40 (each 1H, m, H-1), 1.43 (2H, m, H-2), 3.37 (1H, t, J = 4.5 Hz, H-3), 1.23 (1H, m, H-5), 1.38 (2H, m, H-6), 1.24 & 1.55 (each 1H, m, H-7), 1.42 (1H, m, H-9), 1.52 (2H, m, H-11), 1.53 & 1.91 (each 1H, m, H-12), 1.58 (1H, m, H-13), 1.04 & 1.45 (each 1H, m, H-15), 1.77 (2H, m, H-16), 1.69 (1H, m, H-17), 0.93 (3H, s, H-18), 0.82 (3H, s, H-19), 1.13 (3H, s, H-21), 1.44 (2H, m, H-22), 2.02 (2H, m, H-23), 5.10 (1H, t, J = 5.4 Hz, H-24), 1.66 (3H, s, H-26), 1.59 (3H, s, H-27), 0.91 (3H, s, H-28), 0.81 (3H, s, H-29), 0.86 (3H, s, H-30). 13C-NMR (CDCl3, 125 MHz) δC 33.7 (C-1), 24.9 (C-2), 76.4 (C-3), 37.7 (C-4), 49.6 (C-5), 18.3 (C-6), 35.2 (C-7), 40.7 (C-8), 50.4 (C-9), 37.3 (C-10), 21.4 (C-11), 25.4 (C-12), 42.3 (C-13), 50.5 (C-14), 31.2 (C-15), 27.6 (C-16), 49.8 (C-17), 15.6 (C-18), 16.1 (C-19), 75.5 (C-20), 25.5 (C-21), 40.6 (C-22), 22.6 (C-23), 124.8 (C-24), 131.7 (C-25), 25.9 (C-26), 17.8 (C-27), 28.4 (C-28), 22.2 (C-29), 16.6 (C-30). HR-TOFMS m/z 445.4035 [M+H]+ (calcd. for C30H53O2+, m/z 445.4040). The HR-TOFMS and chemical shift of synthetic analog 4 were similar with 3β,20S-dihydroxy-dammar-24-en-3-on [25]. Therefore, compound 4 was identified as 3β,20S-dihydroxy-dammar-24-en.

The 20S,24S-epoxy-3β,25-dihydroxydammarane (5) were obtained as a colorless crystal. 1H-NMR (CDCl3, 500 MHz) δH 1.42 (2H, m, H-1), 1.55 (2H, m, H-2), 3.38 (1H, t, J = 3.0 Hz, H-3), 1.24 (1H, m, H-5), 1.39 (2H, m, H-6), 1.63 (2H, m, H-7), 1.44 (1H, m, H-9), 1.25 & 1.53 (each 1H, m, H-11), 1.23 & 1.75 (each 1H, m, H-12), 1.62 (1H, m, H-13), 1.04 & 1.40 (each 1H, m, H-15), 1.44 & 1.51 (each 1H, m, H-16), 1.44 (1H, m, H-17), 0.84 (3H, s, H-18), 0.87 (3H, s, H-19), 1.13 (3H, s, H-21), 1.22 (2H, m, H-22), 1.85 (2H, m, H-23), 3.62 (1H, dd, J = 4.8, 10.2 Hz, H-24), 1.17 (3H, s, H-26), 1.09 (3H, s, H-27), 0.92 (3H, s, H-28), 0.82 (3H,
s, H-29), 0.95 (3H, s, H-30). $^{13}$C-NMR (CDCl$_3$, 125 MHz) $\delta$C 33.7 (C-1), 25.4 (C-2), 76.4 (C-3), 37.3 (C-4), 49.6 (C-5), 18.3 (C-6), 34.8 (C-7), 40.7 (C-8), 50.7 (C-9), 37.7 (C-10), 21.7 (C-11), 27.1 (C-12), 42.8 (C-13), 50.2 (C-14), 31.5 (C-15), 25.9 (C-16), 49.8 (C-17), 16.6 (C-18), 16.6 (C-19), 86.7 (C-20), 27.3 (C-21), 35.3 (C-22), 26.4 (C-23), 86.3 (C-24), 70.3 (C-25), 27.9 (C-26), 24.1 (C-27), 28.4 (C-28), 22.2 (C-29), 15.6 (C-30). HR-TOFMS $m/z$ 461.3993 [M+H]$^+$ (calcd. for C$_{30}$H$_{53}$O$_3^+$, m/z 461.3989). The HR-TOFMS and chemical shift of synthetic analog 5 were similar to the 20S,24S-epoxy-3$\beta$,25-dihydroxydammarane [25]. Therefore, compound 5 was identified as the 20S,24S-epoxy-3$\beta$,25-dihydroxydammarane.

2.2. Cytotoxic Activity of All Obtained Compounds

The cytotoxic activity of compounds 1–5 was evaluated against MCF-7 breast cancer cells and B16-F10 melanoma cell lines. Cisplatin was used as a positive control, as shown in Table 2. The experimental result and the statistical graphs of IC$_{50}$ are presented in Figures S19–S28.

Table 2. Cytotoxic activities against MCF-7 and B16F10 cell lines for 1–5.

| Compounds                                                                 | IC$_{50}$ for MCF-7 (µg/mL) | IC$_{50}$ for B16F10 (µg/mL) |
|---------------------------------------------------------------------------|-----------------------------|-----------------------------|
| 3$\beta$-oleate-20S-hydroxydammar-24-en (1)                                 | 313.23                      | 181.34                      |
| 20S,24S-epoxy-3$\beta$-oleate-25-hydroxydammarane (2)                      | 212.21                      | 98.40                       |
| 20S-hydroxydammar-24-en-3-on (3)                                           | 67.30                       | 22.95                       |
| 3$\beta$,20S-dihydroxydammar-24-en (4)                                     | 121.01                      | 49.57                       |
| 20S,24S-epoxy-3$\beta$,25-dihydroxydammarane (5)                           | 82.61                       | 95.27                       |
| Cisplatin (positive control)                                               | 53.00                       | 43.00                       |

The cytotoxic activity against MCF-7 breast cancer and B16-F10 melanoma cells of 1 and 2 was very weak compared with the positive control. This indicates that the fatty acid substituent decreased the cytotoxic activity. Bradley et al. [30] reported that the presence of fatty acid substituents could reduce the toxicity of anticancer drugs. Therefore, this observation was appropriate for the literature.

A literature review on dammarane-type triterpenoid, protopanaxadiol, and protopanaxatriol derivatives with no epoxide ring at the side chain showed high anticancer activity in a previous Chinese patent [31]. This implies that the formation of the epoxide ring decreases anticancer activity. Protopanaxadiol and protopanaxatriol derivatives have similar structural features to compound 3 but contain additional hydroxyl groups in C-12, significantly increasing cytotoxic activity. Many structural features influence cytotoxic activity. Based on the observation of the structure-activity relationship, it can be concluded that the absence of cyclization at the side chain and the presence of the ketone group at C-3, for example, in 3 significantly increases cytotoxic activity for both MCF-7 and B16-F10 cells, indicating that 3 has the highest activity compared to others.

3. Materials and Methods

3.1. General Experimental Procedures

Optical rotations were measured using an ATAGO AP-300 automatic polarimeter (Saitama, Japan), and the high-resolution mass spectra (HRESI-TOFMS) were obtained on a Waters Xevo Q-TOF direct probe/MS system using ESI$^+$ mode and microchannel plates MCPs detector (Milford, MA, USA). In contrast, the IR spectra were measured on a One Perkin Elmer infrared-100 (Shelton, CT, USA). The NMR data were recorded on a JEOL ECZ-500 spectrometer (Tokyo, Japan) at 500 MHz for $^1$H and 125 MHz for $^{13}$C using TMS as the internal standard. Chromatographic separations were conducted on a silica gel G60 (Merck, Darmstadt, Germany, 70–230 and 230–400 mesh), and the TLC plates were precoated with GF$_{254}$ (Merck, 0.25 mm), after which detection was performed by spraying with 10% H$_2$SO$_4$ in ethanol, before heating.
3.2. Plant Material

The stem bark of *A. elliptica* was obtained from the Bogor Botanical Garden, West Java, Indonesia, in June 2015. Subsequently, the plant was identified and classified by the staff of Herbarium Bogoriense, and a voucher specimen with No. Bo-1294562 was deposited at the herbarium.

3.3. Extraction and Isolation

The dried stem bark of *A. elliptica* (2.3 kg) was extracted with methanol (12 L) at room temperature for 5 days. Methanol was used to extract nearly all components from plants because of its ability as a magic solvent. After solvent evaporation, 321.5 g extract was obtained, and partition of methanol extract resulted in 22.6 g, 31.4 g, and 34.5 g of the *n*-hexane, ethyl acetate, and *n*-butanol extract, respectively.

A total of 22.6 g of the *n*-hexane extract was fractionated by vacuum liquid chromatography (r: 5 cm; h: 12 cm) on silica gel (300 g of G60 silica gel) using a gradient elution of *n*-hexane-ethyl acetate (10:0–0:10, stepwise 10%; v: 500 mL) followed by ethyl acetate-methanol (10:0–0:10, stepwise 10%; v: 500 mL) to yield five fractions (A–E). Subsequently, 6 g of C was separated with silica gel column chromatography (70–230 mesh, 63 g) using a gradient elution of *n*-hexane-ethyl acetate (10:0–1:1 stepwise 5%; r: 4 cm; h: 15 cm; v: 400 mL) to yield five fractions (C1–C5). A total of 2.1 g of C3 was separated with silica gel column chromatography (70–230 mesh, 63 g) using a gradient elution of *n*-hexane-ethyl acetate (10:0–7:3 stepwise 2.5%; r: 2 cm; h: 15 cm; v: 240 mL) to produce eight fractions (C3a-C3h). Approximately 300 mg of C3b fraction was separated with silica gel column chromatography (230–400 mesh, 18 g) using *n*-hexane: methylene chloride and ethyl acetate in a ratio of 20:2:1 to yield six fractions (C3b1-C3b6). Moreover, C3b2 produced compound 1 (7 mg), and 73 mg of C3b4 fraction was then separated with silica gel column chromatography (230–400 mesh, 2.8 g) using *n*-hexane-ethyl acetate in a ratio of 20:1 to produce C3b4a and C3b4b. C3b4a also produced compound 2 (8.8 mg), and 516 mg of C3c was separated with silica gel column chromatography (230–400 mesh, 21 g) using *n*-hexane-ethyl acetate in a ratio of 23:2 to yield three fractions, such as C3c1-C3c3. Finally, compound 3 (5 mg) was produced by recrystallizing 50 mg of C3c2.

3β-oleate-205-hydroxydammar-24-en (1): colourless oil; [α]D25 +11.3 (c 0.1, MeOH); IR (KBr) δmax 3490, 2947, 2834, 1729, 2860, 1456, 1375, 1172 cm–1; HR-TOFMS m/z 709.6489 [M+H]+ (calcd. for C48H85O3+, m/z 709.6493); 1H-NMR (CDCl3, 500 MHz) and 13C-NMR (CDCl3, 125 MHz) shown in Table 1.

3β-oleate-205,24S-epoxy-25-hydroxydammarane (2): colourless oil; [α]D25 +15.7 (c 0.1, MeOH); IR (KBr) δmax 3511, 2955, 2853, 1731, 1467, 1454, 1376, 1173 cm–1; HR-TOFMS m/z 725.6444 [M+H]+ (calcd. for C48H85O4+, m/z 725.6442); 1H-NMR (CDCl3, 500 MHz) and 13C-NMR (CDCl3, 125 MHz) shown in Table 1.

3.4. Transesterification of Triterpenoids Fatty Acid Ester

The transesterification of fatty acid triterpene esters 1 and 2 was conducted according to the previously reported procedures [27]. The fatty acid esters at 4 mg each were stirred for 5 h with 0.9 mg sodium methoxide in dry MeOH at 0.5 mL using a magnetic stirrer. Subsequently, the reaction product was extracted with H2O and CHCl3, and the organic phase was separated, dried with Na2SO4, and then concentrated under vacuum to obtain methyl ester of the fatty acid moiety. HCl (1%) was added to the remaining aqueous phase, followed by extraction with CHCl3 to yield triterpenoid alcohol moiety crude. Finally, using silica gel column chromatography with *n*-hexane-ethyl acetate in a ratio of 4:1, the triterpenoid moiety crude was purified to yield triterpenoid moiety each 4 (1 mg) and 5 (1.2 mg).

The mass spectrum recorded of the transesterification product of methyl ester 1 and 2 showed [M+H]+ m/z 297.2777 (calcd. for C19H37O2+, m/z 297.2794) and [M+H]+ m/z 297.2777 (calcd. for C19H37O2+, m/z 297.2794), respectively. This research’s Results and
Discussion section contains the mass spectrum of triterpenoid alcohol moiety of transesterification products 4 and 5, along with NMR data.

3.5. Determination of Cytotoxic Activity

The cytotoxic bioassay was conducted using the PrestoBlue assay, as reported by Izdihar et al. [32]. Presto Blue reagent (Thermo Fisher Scientific, Uppsala, Sweden) was used to quickly and quantitatively analyze the proliferation of different resazurin-based cell types employing live-cell reduction capabilities. Cells maintain a reduced cytosolic environment when they are alive and healthy. Reducing resazurin (blue) works as a cell viability indicator by using absorbance or fluorescent outputs to decrease resorufin (purple). The conversion is proportional to the number of metabolically active cells. Furthermore, the IC_{50} value is the concentration for 50% growth inhibition. The percentage of cytotoxicity compared to untreated cells was determined with the equation below. A plot of % cytotoxicity versus sample concentrations showed 50% cytotoxicity (IC_{50}). Finally, all assays and analyses were performed in duplicate and averaged.

4. Conclusions

Three dammarane-type triterpenoids, including 3β-oleate-20S-hydroxydammar-24-en (1), 3β-oleate-20S,24S-epoxy-25-hydroxydammarane (2), and 20S-hydroxydammar-24-en-3-on (3), were successfully isolated from the stem bark of Aglaia elliptica (C.DC.) Blume. Compound 1 and 2 were elucidated as new dammarane-type triterpenoids fatty acid ester derivatives, whereas 3 was identified as a known compound. The transesterification of 1 and 2 yielded 3β,20S-dihydroxy-dammar-24-en (4) and 20S,24S-epoxy-3β,25-dihydroxydammarane (5). Furthermore, 4 and 5 were identified as known compounds. Using PrestoBlue reagent, compounds (1–5) were tested against MCF-7 breast cancer cell and B16-F10 melanoma cell lines. The results showed that compound 3 had the strongest activity against both cell lines. The presence of fatty acid substituent in 1 and 2 decreased cytotoxic activity, whereas the ketone group in 3 significantly increased the activity against both cell lines. Finally, the formation of a tetrahydrofuran ring appears to reduce the cytotoxic activity against both cell lines.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/molecules27196757/s1, Figure S1: HRTOFMS Spectrum of 1; Figure S2: FTIR Spectrum of 1; Figure S3. 1H-NMR Spectrum of 1 (500 MHz in CDCl_{3}); Figure S4. 13C-NMR Spectrum of 1 (125 MHz in CDCl_{3}); Figure S5. DEPT-135° Spectrum of 1 (125 MHz in CDCl_{3}); Figure S6. HMQC Spectrum of 1; Figure S7. HMBC Spectrum of 1; Figure S8. 1H-1H-COSY Spectrum of 1; Figure S9. NOESY Spectrum of 1; Figure S10. HRTOFMS Spectrum of 2; Figure S11. FTIR Spectrum of 2; Figure S12. 1H-NMR Spectrum of 2 (500 MHz in CDCl_{3}); Figure S13. 13C-NMR Spectrum of 2 (125 MHz in CDCl_{3}); Figure S14. DEPT-135° Spectrum of 2 (125 MHz in CDCl_{3}); Figure S15. HMQC Spectrum of 2; Figure S16. HMBC Spectrum of 2; Figure S17. 1H-1H-COSY Spectrum of 2; Figure S18. NOESY Spectrum of 2; Figure S19. Results of cytotoxic activity of 1 against MCF-7 cell line; Figure S20. Results of cytotoxic activity of 2 against MCF-7 cell line; Figure S21. Results of cytotoxic activity of 3 against MCF-7 cell line; Figure S22. Results of cytotoxic activity of 4 against MCF-7 cell line; Figure S23. Results of cytotoxic activity of 5 against MCF-7 cell line; Figure S24. Results of cytotoxic activity of 1 against B16-F10 cell line; Figure S25. Results of cytotoxic activity of 2 against B16-F10 cell line; Figure S26. Results of cytotoxic activity of 3 against B16-F10 cell line; Figure S27. Results of cytotoxic activity of 4 against B16-F10 cell line; Figure S28. Results of cytotoxic activity of 5 against B16-F10 cell line; Figure S29. Results of cytotoxic activity of 1 against MCF-7 cell line; Figure S30. Results of cytotoxic activity of 2 against MCF-7 cell line; Figure S31. Results of cytotoxic activity of 3 against MCF-7 cell line; Figure S32. Results of cytotoxic activity of 4 against MCF-7 cell line; Figure S33. Results of cytotoxic activity of 5 against MCF-7 cell line; Figure S34. Results of cytotoxic activity of 1 against B16-F10 cell line; Figure S35. Results of cytotoxic activity of 2 against B16-F10 cell line; Figure S36. Results of cytotoxic activity of 3 against B16-F10 cell line; Figure S37. Results of cytotoxic activity of 4 against B16-F10 cell line; Figure S38. Results of cytotoxic activity of 5 against B16-F10 cell line;
References

1. Harneti, D.; Supratman, U. Phytochemistry and biological activities of Aglaia species. Phytochemistry 2021, 181, 112540. [CrossRef] [PubMed]

2. Pannell, C.M. Taxonomic Monograph of the Genus Aglaia Lour. (Meliaceae); Kew Bulletin Additional Series XVI HMSO: Kew, UK, 1992.

3. Heyne, K. The Useful Indonesian Plants, Research and Development Agency; Ministry of Forestry Publisher: Jakarta, Indonesia, 1982; pp. 1029–1031.

4. Cai, X.; Wang, Y.; Zhao, P.; Li, Y.; Luo, X. Dolabellane diterpenoids from Aglaia odorata. Phytochemistry 2010, 71, 1020–1024. [CrossRef] [PubMed]

5. Yodsaoue, O.; Sonprasit, J.; Karalai, C.; Ponglimanont, C.; Tewtrakul, S.; Chantrapromma, S. Diterpenoids and triterpenoids with potential anti-inflammatory activity from the leaves of Aglaia odorata. Phytochemistry 2012, 76, 83–91. [CrossRef] [PubMed]

6. Harneti, D.; Tjokronegoro, R.; Safari, A.; Supratman, U.; Loong, X.M.; Mukhtar, M.R.; Mohamad, K.; Awang, K.; Hayashi, H. Cytotoxic triterpenoids from the bark of Aglaia smithii. Phytochem. Lett. 2012, 5, 496–499. [CrossRef]

7. Su, B.; Chai, H.; Mi, Q.; Riswan, S.; Kardono, L.B.S.; Afriastini, J.J.; Santarsiero, B.D.; Mesecar, A.D.; Fransworth, N.R.; Cordell, G.A.; et al. Activity-guided isolation of cytotoxic constituents from the bark of Aglaia crassinervia collected in Indonesia. J. Bioorg. Med. Chem. 2006, 14, 960–972. [CrossRef]

8. Harneti, D.; Permatasari, A.A.; Anisshabira, A.; Naini, A.A.; Nurlelasari; Mayanti, T.; Maharani, R.; Safari, A.; Hidayat, A.T.; Farabi, K.; et al. Sesquiterpenoids from the stem bark of Aglaia grandis. Nat. Prod. Sci. 2022, 8, 6–12.

9. Joycharat, N.; Plodpai, P.; Panthong, K.; Yingyongnarongkul, B.-E.; Voravuthikunchai, S.P. Terpenoid constituents and antifungal activity of Aglaia forbesii seed against phytopathogens. Can. J. Chem. 2010, 88, 937–944. [CrossRef]

10. Farabi, K.; Harneti, D.; Nurlelasari; Maharani, R.; Hidayat, A.C.; Awang, K.; Supratman, U.; Shiono, Y. New cytotoxic pro-tolimonoids from the stem bark of Aglaia auriclavata (Meliaceae). Phytochem. Lett. 2017, 21, 211–215. [CrossRef]

11. Sun, Y.; Cui, L.; Sun, Y.; Li, Q.; Li, Y.; Wang, Z.; Xu, W.; Kong, L.; Luo, J. A/D-rings-seco limonoids from the fruits of Aglaia edulis and their bioactivities. Phytochemistry 2022, 195, 113049. [CrossRef]

12. Sun, Y.; Cui, L.; Sun, Y.; Li, Q.; Li, Y.; Wang, Z.; Xu, W.; Kong, L.; Luo, J. A/D-rings-seco limonoids from the fruits of Aglaia edulis and their bioactivities. Phytochemistry 2022, 195, 113049. [CrossRef]

13. Wang, X.B.; Wang, H.; Li, Z.R.; Yang, M.H.; Luo, J.; Kong, L.Y. Cytotoxic rocaglate derivatives from leaves of Aglaia perviridis. Sci. Rep. 2016, 28, 20045. [CrossRef] [PubMed]
18. Pan, L.; Acuna, U.M.; Li, J.; Jena, N.; Ninh, T.N.; Pannel, C.M.; Chai, H.; Fuchs, J.R.; Carcache, E.J.; Soejarto, D.D.; et al. Bioactive flavaglines and other constituents isolated from Aglaia perviridis. *J. Nat. Prod.* 2013, 76, 394–404. [CrossRef]

19. Nugroho, B.W.; Gussregen, B.; Wray, V.; Witte, L.; Bringmann, G.; Proksch, P. Insecticidal rocamonalide derivatives from Aglaia elliptica and A. harmsiana. *Phytochemistry* 1997, 45, 1579–1585. [CrossRef]

20. Engelmeier, D.; Hadacek, F.; Pacher, T.; Vajrodaya, S.; Greger, H. Cyclopenta [b] benzofurans from Aglaia species with pronounced antifungal activity against Rice Blast Fungus (Pyricularia grisea). *J. Agric. Food. Chem.* 2000, 48, 1400–1404. [CrossRef]

21. Zhang, H.; Xu, H.H.; Sonf, Z.; Chen, L.Y.; Wen, H.J. Molluscidal activity of Aglaia dupeana and the constituents of its twigs and leaves. *Fitoterapia* 2012, 83, 1081–1086. [CrossRef]

22. Zhang, F.; Wang, J.S.; Gu, Y.C.; Kong, L.Y. Triterpenoids from Aglaia abbreviata and their cytotoxic activities. *J. Nat. Prod.* 2010, 73, 2042–2046. [CrossRef]

23. Harneti, D.; Supriadin, A.; Ulfah, M.; Safari, A.; Supratman, U.; Awang, K.; Hayashi, H. Cytotoxic constituents from the bark of Aglaia eximia (Meliaceae). *Phytochem. Lett.* 2014, 8, 28–31. [CrossRef]

24. Oktaviani, D.; Sukmawati, W.; Farabi, K.; Harneti, D.; Nurlelasari; Darwati; Maharani, R.; Mayanti, T.; Safari, A.; Supratman, U. Triterpenoids from the stem bark of Aglaia eximia (Meliaceae) and their cytotoxic activity against HeLa and DU145 cancer cell lines. *Molekul* 2022, 17, 76–84. [CrossRef]

25. Ruan, J.; Zheng, C.; Qu, L.; Liu, Y.; Han, L.; Yu, H.; Zhang, Y.; Wang, T. Plant resources: 13C-NMR spectral characteristic and pharmacological activities of dammarane-type triterpenoids. *Molecules* 2016, 21, 1047. [CrossRef]

26. Heliawati, L.; Khatimah, H.; Hermawati, E.; Syah, Y.M. Four dammarane triterpenes and their inhibitory properties against eight receptor tyrosine kinases. *Nat. Prod. Sci.* 2020, 26, 345–350.

27. Bradley, O.M.; Webb, N.L.; Antony, F.H.; Devanesan, P.; Witman, P.A.; Hemamalini, S.; Chander, M.C.; Baker, S.D.; He, L.; Horwitz, S.B.; et al. Tumor targeting by covalent conjugation of a natural fatty acid to paclitaxel. *Clin. Cancer Res.* 2001, 7, 3229–3238.

28. Cao, J.; Zhang, X.; Qu, F.; Guo, Z.; Zhao, Y. Dammarane triterpenoids for pharmaceutical use: A patent review (2005–2014). *Expert. Opin. Ther. Pat.* 2015, 25, 805–817. [CrossRef]

29. Izdihar, G.; Naini, A.A.; Harneti, D.; Maharani, R.; Nurlelasari; Mayanti, T.; Safari, A.; Farabi, K.; Supratman, U.; Azmi, M.N.; et al. Sesquiterpenoids from the stem bark of Aglaia simplicifolia and their cytotoxic activity against B16-F10 melanoma skin cancer cell. *Indones. J. Chem.* 2021, 21, 1560–1567. [CrossRef]