NOTE:

Cytotoxic Sesquiterpenoids from the Stem Bark of *Aglaia harmsiana* (Meliaceae)

Hersa Milawati[1], Winda Sukmawati[1], Desi Harneti[1], Rani Maharani[1,2], Nurlelasari[1], Ace Tatang Hidayat[1,2], Darwati[1], Unang Supratman[1,2,*], and Yoshihito Shiono[3]

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang Km. 21, Jatinangor, Sumedang 45363, Indonesia*

[2]*Central Laboratory of Universitas Padjadjaran, Jl. Raya Bandung-Sumedang Km. 21, Jatinangor, Sumedang 45363, Indonesia*

[3]*Department of Food, Life, and Environmental Science, Faculty of Agriculture, Yamagata University, Tsuruoka, Yamagata 997-8555, Japan*

* ***Corresponding author:***

tel: +62-22-7794391
email: unang.supratman@unpad.ac.id

Received: July 18, 2019
Accepted: March 13, 2020

DOI: 10.22146/ijc.47808

Abstract: *Three aromadendrane-type sesquiterpenoids, spathulenol (**1**), 4β,10α-dihydroxyaromadendrane (**2**), and 4α,10α-dihydroxyaromadendrane (**3**) were isolated from the stem bark of* Aglaia harmsiana *(Meliaceae). Compound **3** was isolated for the first time from* Aglaia *genus. The chemical structures of isolated compounds were elucidated by various spectroscopic methods, including one and two-dimensional NMR, as well as mass spectroscopy analyses. These sesquiterpenoids **1**-**3** were evaluated for their cytotoxic activity against MCF-7 breast cancer cell lines. The IC_{50} value of compound **1**-**3** were 31.65 ±0.1, 8.41 ±0.04 and 2.80 ±0.02 μM, respectively. Among the aromadendrane-type sesquiterpenoids, compounds **2** and **3**, which do not have a double bond, showed higher activity than compound **1**. Whereas, compound **3** showed the strongest activity indicate that α configuration of hydroxyl group increases the cytotoxic activity.*

Keywords: Aglaia harmsiana*; cytotoxic activity; aromadendrane-type sesquiterpenoid; MCF-7 cell lines*

■ INTRODUCTION

Sesquiterpenoids are derived from three isoprene units and exist in a wide variety of forms, including linear, monocyclic, bicyclic, and tricyclic frameworks. Sesquiterpenoids are the most diverse group of terpenoids. Most of the sesquiterpenoids (especially hydrocarbons) are considered as essential oil components. They are the principal constituents of cedarwood (98%), vetiver (97%), spikenard (93%), sandalwood (90%), patchouli (71%), myrrh (62%), and ginger (59%) [1].

Sesquiterpenoids can be found in many living system, particularly in higher plants. They arise from common precursor, farnesyl pyrophosphate, by various cyclization reactions [2].

Aromadendrane sesquiterpenoids are the most abundant group of compounds containing *gem*-dimethylcyclopropane rings fused to a hydroazulene skeleton (bicycle [5.3.0]decapentane) [3] and derived from the large group of guaiane sesquiterpenoids with cyclic formation between C-6 and C-11; thus aromadendrane sesquiterpenoids are commonly called 6,11-cycloguaiane [2]. The biological activities of aromadendrane-type sesquiterpenoids have been investigated, including antifeedant, antifouling, antimicrobial, antiviral, insect repellent, and cytotoxic activity [4-5].

Aglaia is the largest genus of the family Meliaceae comprises more than 100 species which are highly distributed throughout the Western Pacific, Indonesia, Malaysia, and India. The bark of these plants has been used as traditional Indonesian herbal medicine for fever treatment, diarrhea, cough, and skin disease for a long time [6-10]. *A. harmsiana* is one of *Aglaia* plants that has

been phytochemically investigated before and it has been proven to contain cycloartane triterpenoids [11-12] and rocaglamide derivative with insecticidal activity [13]. In total, less than ten aromadendrane-type sesquiterpenoids were investigated separately from the *Aglaia* species [14-15], and this type of compound did not attract considerable attention [16].

In order to observe the cytotoxic constituents of Indonesian *Aglaia* plants against MCF-7 breast cancer cell lines, we report the isolation, the structure elucidation, as well as cytotoxic evaluation of three aromadendrane-type sesquiterpenoids **1**-**3** from *A. harmsiana*.

■ EXPERIMENTAL SECTION

Materials

The stem bark of *A. harmsiana* was collected from Bogor Botanical Garden, Bogor, West Java Province, Indonesia, in July 2017. The plant was identified by Mr. Ismail, the staff of Bogoriense Herbarium, Research Center for Biology, Indonesian Institute of Science, Bogor, Indonesia and the voucher specimen has been deposited at the herbarium.

Instrumentation

The IR spectra were recorded on Perkin Elmer Spectrum 100 FT-IR spectrometer using NaCl plate. The mass spectra were determined with Waters Q-TOF Xevo mass spectrometer instrument. The NMR spectrum of compound **1** were recorded on JEOL JNM-ECX500 spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C, and spectra of compound **2**-**3** were recorded on JEOL JNM-ECX500R/S1 spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C with TMS as an internal standard. The column chromatography was performed on silica gel 60 (Merck, 70–230 and 230–400 mesh). The TLC analyses were carried out with silica GF_{254} (Merck, 0.25 mm) using various solvent systems and spot detection was achieved by spraying with 10% H_2SO_4 in EtOH, followed by heating and irradiating under ultraviolet-visible light (254 and 365 nm).

Procedure

Extraction and Isolation

Dried bark *A. harmsiana* (1.7 kg) was extracted with *n*-hexane (12 L) at room temperature. The extract was evaporated by rotary evaporator at 40 °C under reduce pressure to dryness to obtain 43.25 g of concentrated *n*-hexane extract. Then, the dried bark residue was once again extracted with ethyl acetate at room temperature to obtain 43.97 g concentrated ethyl acetate extract. The *n*-hexane extract (43.25 g) was fractionated by vacuum liquid chromatography on silica gel using gradient elution of *n*-hexane:EtOAc:MeOH to afford four subfractions. Fraction B (11.4 g) was further fractionated by vacuum liquid chromatography using gradient elution of *n*-hexane:CH_2Cl_2 to afford nine fractions. Fraction B8 (892 mg) was separated using column chromatography on silica gel eluted with *n*-hexane:EtOAc (4.8:0.2) to give compound **1** (65.4 mg).

The ethyl acetate extract (43.97 g) was fractionated by vacuum liquid chromatography on silica gel using gradient elution of *n*-hexane:EtOAc:MeOH to afford ten subfractions. Fraction F (2.8 g) was further separated using column chromatography on silica gel using gradient elution of *n*-hexane:EtOAc. Fraction F1 (562.3 mg) was separated using column chromatography on silica gel which eluted with a gradient of *n*-hexane:$CHCl_3$ to give compound **2** (11.3 mg). Fraction F3 (407.3 mg) was separated on silica gel using column chromatography using gradient elution of *n*-hexane:$CHCl_3$ to afford fraction F3C. Then, fraction F3C (115.3 mg) was separated by RP-18 silica gel column chromatography using gradient elution with MeOH:H_2O to give compound **3** (10.5 mg).

Spathulenol (1). Colorless oil, IR (NaCl) ν_{max} 3434; 3065; 2970; 1671; 1385; 1365; 1163 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz), ^{13}C-NMR ($CDCl_3$, 125 MHz) see Table 1. HR-TOFMS *m/z* found 221.1813 $[M+H]^-$, (calculated for $C_{15}H_{25}O$, *m/z* 221.1905).

4β,10α-dihydroxyaromadendrane (2). Colorless oil, IR (NaCl) ν_{max} 3301; 2872; 1457; 1381; 1360; 968 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz), ^{13}C-NMR ($CDCl_3$, 125 MHz) see Table 1. HR-TOFMS *m/z* found 237.1823 $[M-H]^-$, (calculated for $C_{15}H_{25}O_2$, *m/z* 237.1855).

4α,10α-dihydroxyaromadendrane (3). Colorless oil, IR (NaCl) ν_{max} 3428; 2868; 1465; 1382; 1379; 1051 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz), ^{13}C-NMR ($CDCl_3$, 125

MHz) see Table 1. HR-TOFMS *m/z* found 261.1857 $[M+Na]^+$, (calculated for $C_{15}H_{25}O_2Na$, *m/z* 261.1831).

In vitro *cytotoxic activity assay*

Cell viability was assessed by PrestoBlue reagent (Thermo Fisher Scientific, Uppsala, Sweden) based on the reduction of resazurin (blue), which works as a cell viability indicator, to resofurin (purple). PrestoBlue can be evaluated quantitatively visually using absorbance or utilizing the fluorescent outputs of the reduced resofurin. The conversion is proportional to the number of metabolically active cells. The MCF-7 cells were used for *in vitro* breast cancer studies because they retained several particular properties to mammary epithelium, such as the processing of estrogen, in the form of estradiol, via estrogen receptors (ER) in the cell cytoplasm [17]. Briefly, MCF-7 cell lines were grown in 70% confluent were harvested and counted, then diluted with complete culture RPMI medium (Gibco Thermo, Uppsala, Sweden). The cells were then transferred into 96 well-plates with a total of 17×10^3 cells/well. After overnight growth, the cells were treated with increasing concentrations of compounds **1**-**3** (7.8, 15.6, 31.25, 62.5, 125, 250, 500, 1000 ppm) with co-solvent with various percentages of DMSO (0.5–2.5%) in PBS (Uppsala, Sweden). Cisplatin (Sigma Aldrich, USA) was used as the positive control. All samples were incubated at 37 °C in a 5% CO_2 incubator for 24 h. After incubation, the medium was immediately replaced by 10 µL PrestoBlue reagent in 90 µL RPMI medium. The plates were incubated for 1–2 h until resofurin was formed (color changes from blue to purple). The absorbance was measured at 570 nm using a microplate reader. The IC_{50} value is the required concentration for 50% growth inhibition. The percentage of cytotoxicity compared to untreated cells was determined with the equation given below. A plot of % cytotoxicity versus sample concentrations was used to calculate the concentration which showed 50% cytotoxicity (IC_{50}) [18-19]. All assay and analyses were each run in duplicate and all were averaged.

$$\text{Cytotoxicity } (\%) = \frac{[(A_1 - A_2) \times 100]}{A_1}$$

A_1 = Absorbance of untreated group

A_2 = Absorbance of treated group

■ RESULTS AND DISCUSSION

The *n*-hexane and ethyl acetate extract of the stem bark of *A. harmsiana* was subjected to repeated column chromatography to afford three aromadendrane-type sesquiterpenoids. The isolated compounds were elucidated as known compounds namely spathulenol (**1**), 4β,10α-dihydroxyaromadendrane (**2**), and 4α,10α-dihydroxyaromadendrane (**3**) (Fig. 1), by interpreting spectral data and making comparisons with literature values. All these three compounds were reported from *A. harmsiana* for the first time while 4α,10α-dihydroxyaromadendrane (**3**) itself was isolated from genus *Aglaia* for the first time.

Compound **1** was obtained as a colorless oil. The molecular formula was established by HR-TOF-MS as $C_{15}H_{24}O$ from the molecular ion peak of 221.1813 ($[M+H]^-$ calculated $C_{15}H_{25}O$ 221.1905) in the HR-TOF-MS (Fig. S8), indicating four degrees of unsaturation. The IR spectrum (Fig. S9), showed the absorption bands of hydroxyl group (3434 cm^{-1}) and olefinic bond (1671 cm^{-1}). The ^{1}H-NMR spectrum (Fig. S1), showed the presence of three tertiary methyls at δ_H 1.03 (CH_3-12), 1.28 (CH_3-15) and 1.05 (CH_3-13), and two olefinic protons at δ_H 4.65 (H-14) and 4.67 (H-14). The DEPT and HMQC experiments (Fig. S2-S4), could differentiate

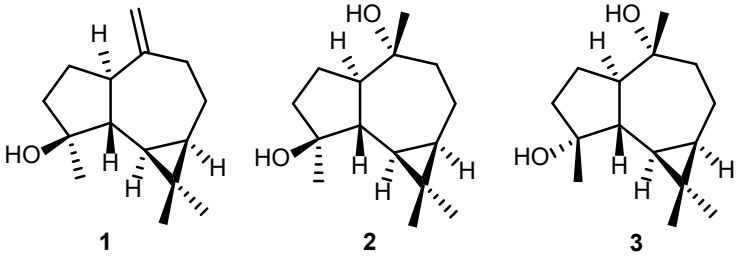

Fig 1. Structures of compound **1**-**3**

the 15 carbon in the ^{13}C-NMR into three methyls, five methylenes (including one olefinic methylene), four methines, and three quaternary carbons (including one olefinic quaternary and one oxygenated quaternary carbon). The structural units of C-3, C-2, C-1, C-5, C-6, C-7, C-8, and C-9 were undoubtedly determined from the ^{1}H-^{1}H-COSY spectrum (Fig. S5 and Fig. 2) which suggested that all aliphatic methine and methylene protons were part of a contiguous spin system comprising H-3, H-2, H-1, H-5, H-6, H-7, H-8, H-9 in the molecules. The fragments of C-1, C-10 (C-14), C-9 and C-3, C-4 (C-15), C-5 were determined by the HMBC strong correlations (Fig. S6) from H-14 to C-1/C-10/C-9 and H-15 to C-3/C-4/C-5, respectively. Additionally, the HMBC correlations from H-5 to C-1/C-6 and the support data from ^{1}H-^{1}H-COSY allowed the definition of the cycloheptane unit. The HMBC correlations from H-12/H-13 to C-11/C-6/C-7 indicated the existence of an isopropyl group located at cyclopropane ring. The NOESY correlations (Fig. S7) of H-1 (α-oriented) with

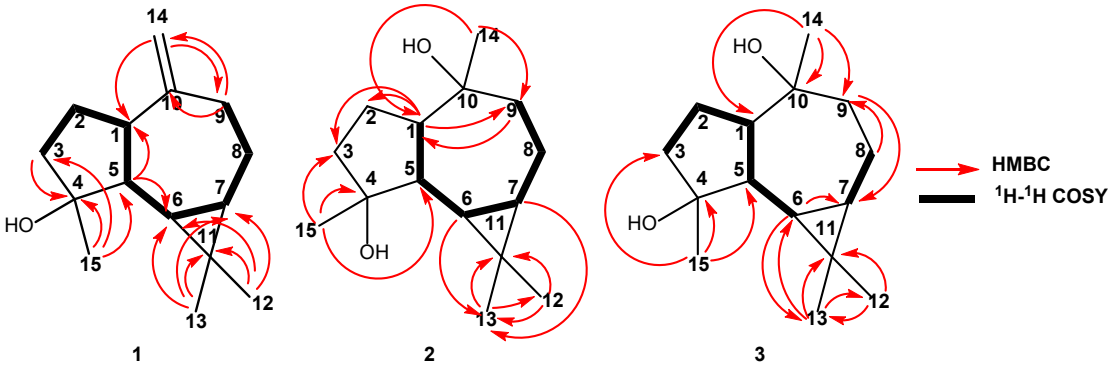

Fig 2. Selected HMBC and ^{1}H-^{1}H-COSY correlations of compound **1**-**3**

Table 1. NMR data of compound **1**-**3** (measured in $CDCl_3$ at 500 MHz and 125 MHz)

Pos.	Compound **1**		Compound **2**		Compound **3**	
	δ_C (mult.)	δ_H (ΣH, mult., *J* = Hz)	δ_C(mult.)	δ_H (ΣH, mult., *J* = Hz)	δ_C(mult.)	δ_H (ΣH, mult., *J* = Hz)
1	53.5 (d)	1.30 (1H, m)	56.4 (d)	1.84 (1H, m)	54.7 (d)	2.14 (1H, m)
2	26.8 (t)	1.89 (1H, dd, 6, 12) 1.62 (1H, dd, 6, 12)	23.8 (t)	1.56 (2H, m)	23.8 (t)	1.90 (1H, m) 1.63 (1H, m)
3	41.8 (t)	1.75 (1H, m) 1.55 (1H, m)	41.2 (t)	1.67 (2H, m)	40.4 (t)	1.44 (1H, m) 1.67 (1H, m)
4	81.1 (s)	-	80.4 (s)	-	80.4 (s)	-
5	54.5 (d)	1.30 (1H, m)	48.5 (d)	1.19 (1H, m)	47.6 (d)	0.91 (1H, m)
6	29.9 (d)	0.45 (1H, dd, 9.0, 10.8)	28.3 (d)	0.41 (1H, t, 10.2)	25.1 (d)	0.63 (1H, m)
7	27.5 (d)	0.70 (1H, m)	26.6 (d)	0.63 (1H, m)	26.2 (d)	0.67 (1H, m)
8	24.8 (t)	1.96 (2H, m)	20.2 (t)	0.89 (1H, m) 2.05 (1H, m)	20.5 (t)	1.83 (2H, m)
9	38.9 (t)	2.41 (1H, dd, 6, 13.8) 2.04 (1H, dd, 6, 13.8)	44.5 (t)	1.5 (1H, m) 1.7 (1H, m)	44.3 (t)	1.5 (1H, m) 1.72 (1H, m)
10	153.5 (s)	-	75.1 (s)	-	75.7 (s)	-
11	20.4 (s)	-	19.6 (s)	-	19.1 (s)	-
12	16.4 (q)	1.02 (3H, s)	16.5 (q)	1.02 (3H, s)	16.5 (q)	0.95 (3H, s)
13	28.7 (q)	1.04 (3H, s)	28.7 (q)	1.02 (3H, s)	29.0 (q)	1.03 (3H, s)
14	106.4 (t)	4.65 (1H, s) 4.67 (1H, s)	20.4 (q)	1.15 (3H, s)	19.8 (q)	1.07 (3H, s)
15	26.2 (q)	1.27 (3H, s)	24.5 (q)	1.23 (3H, s)	25.8 (q)	1.22 (3H, s)

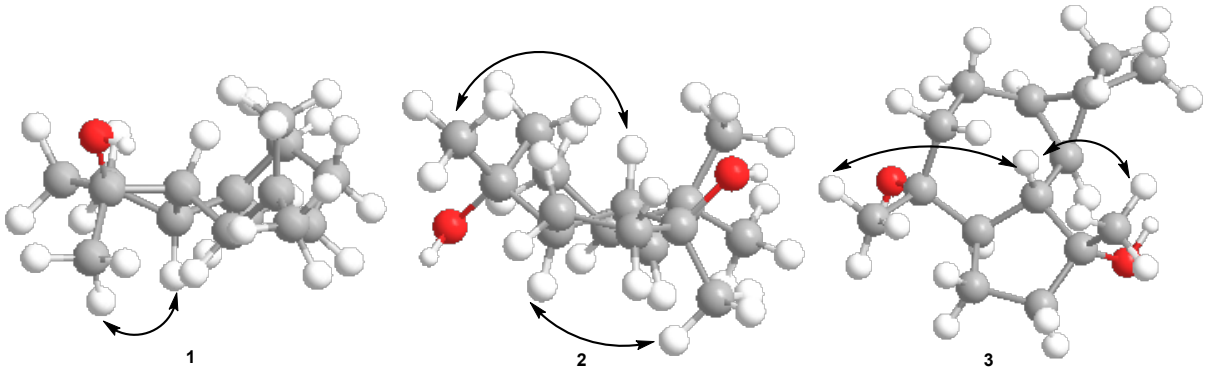

Fig 3. NOE correlations of compound **1**-**3**

H_3-15 suggested CH_3-15 was α-oriented (Fig. 3). The detailed examination of the NMR data and comparison with those reported for spathulenol [4-5] showed that the structures of these two compounds are very similar. Therefore, the structure of compound **1** was determined to be spathulenol.

Compound **2** was obtained as a colorless oil. The molecular formula was established by HR-TOF-MS (Fig. S17) as $C_{15}H_{26}O_2$ from a molecular ion peak of 237.1823 ($[M-H]^+$ calculated $C_{15}H_{25}O_2$ 237.1855) in the HR-TOF-MS, indicating three degrees of unsaturation. The IR spectrum (Fig. S18), showed absorption band of hydroxyl groups at 3301 cm^{-1}.

The ^{1}H-NMR spectrum of **2** (Fig. S10), showed signals assignable for four tertiary methyls at δ_H 1.02 (∑H = 6H, CH_3-12/CH_3-13), 1.15 (CH_3-14) and 1.23 (CH_3-15). The DEPT and HMQC experiments (Fig. S12-S13) of the 15 carbon resonances in the ^{13}C-NMR (Fig. S11) into four methyls, four methylenes, four methines, and three quaternary carbons (including two oxygenated quaternary carbons). The structural units of C-1, C-5, C-6, C-7, C-8, and C-9 were undoubtedly determined from the ^{1}H-^{1}H-COSY spectrum (Fig. 2 and Fig. S14) which suggested that aliphatic methine and methylene protons were part of a contiguous spin system comprising H-1, H-5, H-6, H-7, H-8, and H-9. Then the fragments of C-1, C-10 (C-14), C-9 were determined by the HMBC strong correlations (Fig. S15) from H-14 to C-1/C-10/C-9 allowed the definition of the cycloheptane unit in the molecules. The other methyl group was determined by the strong correlation between H-15 to C-3/C-4/C-5. The cyclopentane ring was supported by the HMBC correlations from H-1 to C-2/C-3. The last two methyl groups were determined by the strong correlation from H-12 to C-13/C-11, H-13 to C-12/C-11, and H-6/H-7 to C-13, which indicated the existence of an isopropyl group located at C-11 to form cyclopropane ring which bound to cycloheptane ring. The NOESY correlations (Fig. S16) of H-1 (α-oriented) with H_3-14 and H-5 (β-oriented) with H_3-15 suggested CH_3-14 was α-oriented while CH_3-15 was β-oriented (Fig. 3). The detailed examination of the NMR spectral data and comparison with those reported for 4β,10α-dihydroxyaromadendrane [14,16] showed that the structures of these two compounds are very similar. Therefore, the structure of compound **2** was determined to be 4β,10α-dihydroxyaromadendrane.

Compound **3** was obtained as a colorless oil. The molecular formula was established by HR-TOF-MS (Fig. S26) as $C_{15}H_{26}O_2$ from a molecular ion peak of 261.1857 ($[M+Na]^+$ calculated $C_{15}H_{26}O_2Na$ 261.1831, indicating three degrees of unsaturation. The IR spectrum showed (Fig. S27) absorption band of hydroxyl groups at 3428 cm^{-1}. The ^{1}H and ^{13}C-NMR spectra of compound **3** (Fig. S19-S20) have a very similar pattern to those of compound **2**, suggested that compounds **2** and **3** have the same functional group and skeleton yet different relative configuration. The ^{1}H-NMR spectrum compound **3** showed signals assignable for four tertiary methyls at δ_H 0.95 (CH_3-12), 1.03 (CH_3-13), 1.07 (CH_3-14) and 1.22 (CH_3-15). The DEPT and HMQC experiments (Fig. S21-S22) of the 15 carbon resonances in the ^{13}C-NMR into four methyls, four methylenes, four methines, and three quaternary carbons (including two oxygenated quaternary carbons). The structural units of C-3, C-2,

C-1, C-5, C-6 along with C-7 and C-8 were undoubtedly determined from the ^{1}H-^{1}H-COSY spectrum (Fig. 2 and Fig. S23) which suggested that aliphatic methine and methylene protons were part of a contiguous spin system comprising H-3, H-2, H-1, H-5, H-6, H-7 and H-8. Then the fragments of C-1, C-10 (C-14), C-9 were determined by the HMBC strong correlations (Fig. S24) from H-14 to C-1/C-10/C-9, and the correlations between H-8 to C-9 and H-9 to C-7 allowed the definition of the cycloheptane unit in the molecules. The other methyl group was determined by the strong correlation between H-15 to C-3/C-4/C-5, which allowed the cyclopentane ring among C-1, C-2, C-3, C-4, and C-5. The last two methyl groups were determined by the strong correlations from H-12 to C-13/C-11, H-13 to C-12/C-11/C-6, and H-6 to C-13/C-7, which indicated the existence of an isopropyl group located at C-11 to form cyclopropane ring which bound to cycloheptane ring. The NOESY correlations (Fig. S25) of H-5 (β-oriented) with H_3-14 and H_3-15 suggested CH_3-14 and CH_3-15 was β-oriented (Fig. 3). The detailed examination of the NMR spectral data and comparison with those reported for 4α,10α-dihydroxyaromadendrane [14,16] showed that the structures of these two compounds are very similar. Therefore, the structure of compound **3** was determined to be 4α,10α-dihydroxyaromadendrane.

The cytotoxic activity of the isolated compounds **1**-**3** were evaluated against the MCF-7 breast cancer cell lines according to a method described in Xu et al. [20]. Cisplatin (IC_{50} = 53 μM, the authors performed the experiments by themselves) was used as a positive control. The results are shown in Table 2. Among all aromadendrane-type sesquiterpenoid compounds, 4α,10α-dihydroxyaromadendrane (**3**) showed the highest cytotoxic activity while spathulenol (**1**) showed the lowest activity. These IC_{50} values indicated that the presence of an olefinic group significantly decreasing the cytotoxic activity, while compared to compound **2**, the α-oriented hydroxyl group increasing the cytotoxic activity. These results suggested that the relative configuration of hydroxyl group and olefinic group played some important structural features for cytotoxic activity in aromadendrane-type sesquiterpenoid.

Table 2. Cytotoxic activity of compound **1**-**3** against MCF-7 breast cancer cell lines

Compounds	IC_{50} (μM)
Spathulenol (**1**)	31.65 ± 0.1
4β,10α-dihydroxyaromadendrane (**2**)	8.41 ± 0.04
4α,10α-dihydroxyaromadendrane (**3**)	2.80 ± 0.02
Cisplatin	53

■ CONCLUSION

Three aromadendrane-type sesquiter-penoid compounds, spathulenol (**1**), 4β,10α-dihydroxyaroma dendrane (**2**), and 4α,10α-dihydroxyaromadendrane (**3**) were isolated from *n*-hexane and ethyl acetate extract of the stem bark of *A. harmsiana*. All these three compounds were reported from *A. harmsiana* for the first time while 4α,10α-dihydroxyaromadendrane (**3**) itself was isolated from genus *Aglaia* for the first time. Compound **1**-**3** were evaluated for their cytotoxic activity against MCF-7 breast cancer cell lines. Among the aromadendrane-type sesquiterpenoids, compound **3** showed the highest activity because the α-oriented hydroxyl group and compound **1** showed the lowest activity because of the presence of an olefinic group, indicated that the relative configuration of the hydroxyl group and the olefinic group played some important structural features for cytotoxic activity in aromadendrane-type sesquiterpenoid.

■ ACKNOWLEDGMENTS

This research was financially supported by the Universitas Padjadjaran, Indonesia (Academic Leadership Grant 2019 by Unang Supratman, No: 3329/UN6.D/LT/2029).

■ REFERENCES

[1] Ludwiczuk, A., Skalicka-Woźniak, K., and Georgiev, M.I., 2017, "Terpenoids" in *Pharmacognosy*, Eds. Badal, S., and Delgoda, R., Academic Press, London, United Kingdom, 233–266.

[2] Connolly, J.D., and Hill, R.A., 1991, *Dictionary of Terpenoids*, Chapman & Hall, London.

[3] Durán-Peña, M.J., Botubol Ares, J.M., Hanson, J.R., Collado, I.G., and Hernández-Galán, R., 2015, Biological activity of natural sesquiterpenoids

containing a *gem*-dimethylcyclopropane unit, *Nat. Prod. Rep*., 32 (8), 1236–1248.

[4] Joycharat, N., Plodpai, P., Panthong, K., Yingyongnarongkul, B., and Voravuthikunchai, S.P., 2010, Terpenoid constituents and antifungal activity of *Aglaia forbessi* seed against phytopathogens, *Can. J. Chem*., 88 (9), 937–944.

[5] Milawati, H., Harneti, D., Maharani, R., Nurlelasari, Hidayat, A.T., Azmi, M.N., Shiono, Y., and Supratman, U., 2019, Caryophyllen-type sesquiterpenoids from the stembark of *Aglaia harmsiana* and their cytotoxic activity against MCF-7 breast cancer cells, *Molekul*, 14 (2), 126–132.

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

[7] Sianturi, J., Purnamasari, M., Darwati, Harneti, D., Mayanti, T., Supratman, U., Awang, K., and Hayashi, H., 2015, New bisamide compounds from the bark of *Aglaia eximia* (Meliaceae), *Phytochem. Lett*., 13, 297–301.

[8] Awang, K., Loong, X.M., Leong, K.H., Supratman, U., Litaudon, M., Mukhtar, M.R., and Mohamad, K., 2012, Triterpenes and steroids from the leaves of *Aglaia exima* (Meliaceae), *Fitoterapia*, 83 (8), 1391–1395.

[9] Hidayat, A.C., Farabi, K., Harneti, D., Nurlelasari, Maharani, R., Nurfarida, I., Supratman, U., and Shiono, Y., 2018, Cytotoxic triterpenoids from the stembark of *Aglaia argentea* (Meliaceae), *Indones. J. Chem*., 18 (1), 35–42.

[10] Farabi, K., Harneti, D., Nurlelasari, Maharani, R., Hidayat, A.T., Awang, K., Supratman, U., and Shiono, Y., 2017, New cytotoxic protolimonoids from the stem bark of *Aglaia argentea* (Meliaceae), *Phytochem. Lett*., 21, 211–215.

[11] Inada, A., Murayta, H., Inatomi, Y., Nakanishi, T., and Darnaedi, D., 1995, Cycloartane triterpenes from the leaves of *Aglaia harmsiana*, *J. Nat. Prod.*, 58 (7), 1143–1146.

[12] Inada, A., Ohtsuki, S., Sorano, T., Murata, H., Inatomi, Y., Darnaedi, D., and Nakanishi, T., 1997, Cycloartane triterpenoids from *Aglaia harmsiana*, *Phytochemistry*, 46 (2), 379–381.

[13] Nugroho, B.W., Gussregen, B., Wray, V., Witte, L., Bringmann, G., and Proksch, P., 1997, Insecticidal rocaglamida derivative from *Aglaia elliptica* and *A. harmsiana*, *Phytochemistry*, 45 (8), 1579–1585.

[14] Kurniasih, N., Milawati, H., Fajar, M., Hidayat, A.T., Abdullah, R., Harneti, D., Supratman, U., and Azmi, M.N., 2018, Sesquiterpenoids compounds from the stembark of *Aglaia minahassae* (Meliaceae), *Molekul*, 13 (1), 56–62.

[15] Inada, A., Shono, K., Murata, H., Inatomi, Y., Darnaedi, D., and Nakanishi, T., 2000, Three putrescine bisamides from the leaves of *Aglaia grandis*, *Phytochemistry*, 53 (8), 1091–1095.

[16] Liu, S., Liu, S.B., Zuo, W.J., Guo, Z.K., Mei, W.L., and Dai, H.F., 2014, New sesquiterpenoids from *Aglaia odorata* var. *microphyllina* and their cytotoxic activity, *Fitoterapia*, 92, 93–99.

[17] Vieira, I.J.C., Figueiredo, E.R., Freitas, V.R., Mathias, L., Braz-Filho, R., and Araujo, R.M., 2010, A new sesquiterpene from *Trichilia casaretii* (Meliaceae), *Am. J. Anal. Chem.*, 2, 70–72.

[18] Camarillo, I.G., Xiao, F., Madhivanan, S., Salameh, T., Nichols, M., Reece, L.M., Leary, J.F., Otto, K.J., Natarajan, A., Ramesh, A., and Sundararajan, R., 2014, "Low and high voltage electrochemotherapy for breast cancer: An *in vitro* model study" in *Electroporation-Based Therapies for Cancer*, Eds. Sundararajan, R., Woodhead Publishing, Cambridge, United Kingdom, 55–102.

[19] Machana, S., Weerapreeyakul, N., Barusrux, S., Nonpunya, A., Sripanidkulchai, B., and Thitimetharoch, T., 2011, Cytotoxic and apoptotic effect of six herbal plants against the human hepatocarcinoma (HepG2) cell line, *Chin. Med.*, 6 (39), 1–8.

[20] Xu, M., McCanna, D.J., and Sivak, J.G., 2015, Use the viability reagent PrestoBlue in comparison with alamarBlue and MTT to assess the viability of human corneal epithelial cells, *J. Pharmacol. Toxicol. Methods*, 71, 1–7.