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A Cycloartane Triterpenoid and Steroid from The Leaves of *Aglaia shawiana* Merr. (Meliaceae)

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Abstract

Natural products and their structural analogs are the main sources of pharmacotherapy. Various *Aglaia* species have been investigated since the 1960s for their phytochemical constituents and biological properties. This research focuses on isolating the terpenoid group from *Aglaia shawiana*, to provide a basis for further research to identify lead compounds for drug development. The hexane extract of *Aglaia shawiana* leaves was isolated using vacuum liquid chromatography and gravity column chromatography, employing solvents with varying polarities. The isolation process yielded two compounds: compound **1** and compound **2**. A total of 15 mg of a pure isolate was obtained for compound **1**, and 4.5 mg for compound **2**. Their structures were characterized using 1D NMR (^{1}H, ^{13}C, DEPT 135) and 2D NMR (HMQC, HMBC, ^{1}H-^{1}H COSY) and by comparing them with previously reported spectral data. It was concluded that compound **1** has the molecular formula $C_{30}H_{50}O_3$, is a triterpenoid type cycloartane, and is named cycloartan-3β,29-diol-24-one, while compound **2** has the molecular formula $C_{30}H_{52}O$, is a steroid compound, and is named 23a-homostigmast-5en-3β-ol. These compounds were discovered for the first time in this species.

1. Introduction

The genus *Aglaia*, the largest genus of subtropical and tropical angiosperms in the Meliaceae family, comprises over 150 species widely distributed across Southern mainland China, the Indo-Malaysian region, and the Pacific Islands [1, 2]. The leaves of these species have long been used in Indonesian herbal medicine to treat fever, diarrhea, infected wounds, coughs, and skin diseases [3]. Based on the literature from 1965 to 2020, around 291 pure compounds have been isolated from the genus *Aglaia*, categorized as sesquiterpenoids (34%), diterpenoids (2%), triterpenoids (33%), limonoids (1%), steroids (10%), lignans (4%), alkaloids (8%), and the flavagline group (34%) [2].

In our continuous search for anticancer candidate compounds from Indonesian *Aglaia* plants, we have previously isolated several cytotoxic compounds from species such as *A. smithii* [4], *A. eximia* [5], *A. argentea* [6], and recently, *A. angustifolia* [7, 8]. However, until now, there have been no reports of the isolation of compounds from the species *Aglaia shawiana* Merr. Commonly found triterpenoid compounds in the genus *Aglaia* include the dammarane type [9] and cycloartane [10], while stigmasterol-type steroid compounds are also prevalent [11]. Both triterpenoid and steroid compounds are known for their significant biological activities [12, 13]. Based on the very interesting potential of triterpenoid and steroid compounds from the Genus *Aglaia*, we continued to isolate these compounds from the leaves of *Aglaia shawiana* Merr. This paper reports the isolation and structural elucidation of a cycloartane-type triterpenoid (1) and a steroid compound (2) from the *n*-hexane leaf extract of *Aglaia shawiana* Merr.

2. Experimental

2.1. Materials

Chromatographic separations were carried out on silica gel 60 (Merck Kieselgel 60 PF 253 Art No. 7734.1000 and 9385.1000 with the particle size 0.063-0.200 mm and 0.040 – 0.063 mm), respectively) and Octa Dodecyl Silane (ODS Fuji Sylisia, Japan). Thin Layer Chromatography (TLC) plates were precoated with silica gel GF_{254} (Merck, 0.25 mm, Darmstadt, Germany). Spots were visualized under UV light of 254 nm and 365 nm simultaneously and by spraying with 20% H_2SO_4 in ethanol followed by heating. 1D NMR spectra (^1H, ^{13}C, and DEPT) and 2D spectra (COSY, HSQC, HMBC, and NOESY) spectra were recorded with a BRUKER AVANCE-700 (700 MHz) (at 700 MHz for ^1H and 125 MHz for ^{13}C-NMR for compounds **1** and **2**, with $CDCl_3$ as a solvent, the chemical shift was given on a δ (ppm) scale and both using tetramethyl silane (TMS) as the internal standard. Technical solvents were distilled before maceration; isolation and spectral grade solvents (*n*-hexane, ethyl acetate, methanol, and dimethylene chloride from Merck, Darmstadt, Germany) were employed for spectroscopic measurements.

2.2. Collection and Preparation of Plant Material

The leaves of *Aglaia shawiana* were collected from Bogor Botanical Garden, Latitude: -6° 35' 30.59" S Longitude: 106 47' 32.39" E, West Java, Indonesia, on July 2022. The following is the taxonomy of this species: kingdom: Plantae, subkingdom: Tracheobionta, superdivision: Spermatophyta, division: Mangnoliophyta, class: Mangnoliopsida, subclass: Rosidae, order: Sapindales, family: Meliaceae, genus: *Aglaia*, species: *Aglaia shawiana* Merr. The plant was identified by the Center for Plant Conservation Botanic Gardens Bogor, Indonesia, and a voucher specimen (II.K.59) was deposited at the Herbarium. in Bogor Botanical Garden, Bogor, West Java Province, Indonesia.

2.3. Extraction of Phytochemicals

The *n*-hexane extract (20.0 g) was subjected to vacuum liquid chromatography (VLC) over silica gel using a gradient elution mixture of *n*-hexane-ethyl acetate (10:0 to 0:10), resulting in nine fractions (A-I). Fraction H (4.4962 g) was further purified using column chromatography (CC) over silica gel (200 mesh) with a gradient elution of *n*-hexane-ethyl acetate (10:0 to 8:2), followed by another gradient elution (8:2 to 0:10), yielding 16 sub-fractions (H1-H16). Sub-fraction H6 (4.1560 g) underwent CC over silica gel (200 mesh) with an isocratic elution mixture of *n*-hexane-ethyl acetate (7:3), producing 14 sub-fractions (H6A-H6N). Sub-fraction H6C (3.7465 g) was further subjected to CC over silica gel (200 mesh) with the same isocratic elution mixture (7:3), resulting in 14 sub-fractions (H6C1-H6C14).

Furthermore, sub-fractions H6C7 and H6C8 were subjected to CC over silica gel (400 mesh) using an isocratic mixture of *n*-hexane-ethyl acetate (4:6), producing 12 sub-fractions. Sub-fraction H6C7B was purified using CC over octadecyl silica with an isocratic methanol-water mixture (9.8:0.2), yielding compound **1** (15 mg). The purity of isolate 1 was verified using TLC with various eluents, and the spot of compound **1** on the TLC plate had an Rf value of 0.45 with an eluent composition of *n*-hexane-ethyl acetate (5:5). Meanwhile, sub-fraction H6C4 was subjected to CC over silica gel (400 mesh) with an isocratic mixture of *n*-hexane-ethyl acetate (7:3), resulting in eight sub-fractions. Sub-fraction H6C7C was then purified using CC over octadecyl silica with an isocratic methanol-water mixture (9.8:0.2), yielding compound **2** (4.5 mg). The purity of isolate 2 was confirmed using TLC with various eluents, and the spot of compound **2** on the TLC plate had an Rf value of 0.6 with an eluent composition of *n*-hexane-ethyl acetate (7:3).

3. Results and Discussion

3.1. Compound 1

Compound **1** was obtained as a white amorphous powder with the molecular formula $C_{30}H_{50}O_3$. Its molecular composition was established from NMR data. The Hydrogen Deficiency (HD) index was calculated using the equation HD = ΣC - ΣH/2 + 1, yielding an HD index of six for compound **1**. The ^1H NMR ($CDCl_3$, 700 MHz, ppm) spectrum of **1** displayed the presence of three tertiary methyl groups at δ_H 0.88 (3H, *s*, Me-18), 0.95 (3H, *s*, Me-28), and 0.93 (3H, *s*, Me-30), as well as three secondary methyl groups at δ_H 0.81 (3H, d, *J* = 6.0 Hz, Me-21), 1.08 (3H, d, *J* = 7.1 Hz, Me-26), and 1.07 (3H, d, *J* = 7.1 Hz, Me-27). The oxygenated methine and methylene signals were also observed in ^1H-NMR at δ_H 3.78 (1H, dd, 16.9, 11.8 Hz, H-3) and 3.76 (H, dd, 16.9, 11.8 Hz, H-29), and δ_H 3.51 (1H, d; 10.4 Hz, H-29). Singlets spectrum is also seen at δ_H 0.37 (1H, *s*, H-19) and 0.58 (1H, *s*, H-19), which are characteristic of non-equivalent protons on a cyclopropyl methylene group, as depicted in Figure 1.

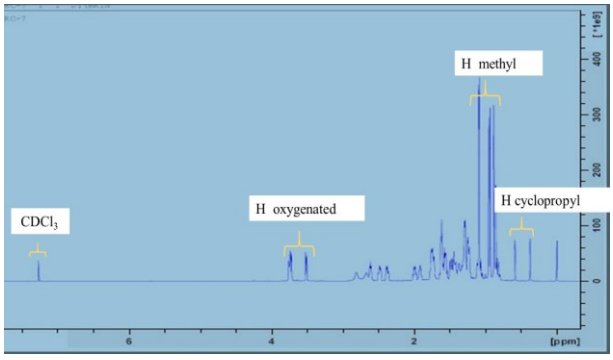

Figure 1. ^1H NMR spectrum of compound **1**

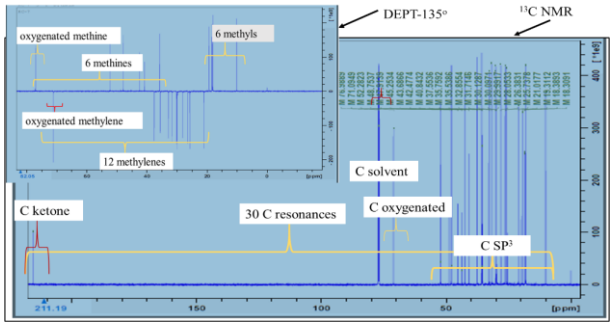

Figure 2. ^{13}C and DEPT-135 NMR spectrum of compound **1**

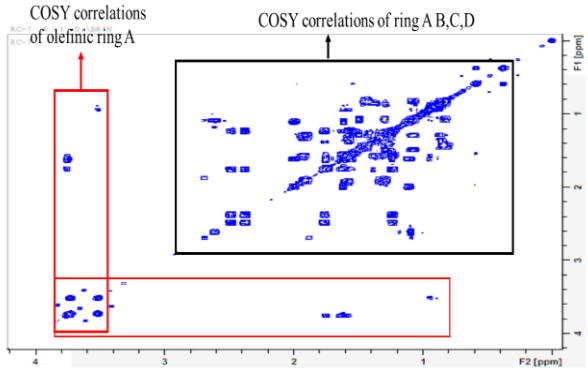

Figure 3. ^{1}H-^{1}H COSY NMR spectrum of compound **1**

The ^{13}C NMR ($CDCl_3$, 175 MHz, ppm) spectrum, as seen in Figure 2, showed 30 carbon resonances. These resonances were classified by their chemical shifts, DEPT, and HMQC spectra as follows: six methyl groups (three tertiary at δ_C 18.4, 19.4, and 10.3, three secondary at δ_C 18.5, 18.2, and 18.2. Twelve methylene groups were found at δ_C 30.2, 30.2, 21.1, 28.2, 25.9, 35.7, 33.0, 26.5, 30.1, 31.8, and 37.7, and one oxygenated methylene at 71.2. Six methine groups were found at δ_C 42.6, 48.0, 52.4, 35.9, 41.0, and an oxygenated methine at 77.1. Six quaternary carbons were found at δ_C 43.8, 20.0, 25.5, 45.4, and 48.9, and a ketone functional carbon group at δ_C 215.7. These functionalities, which account for one out of the six degrees of unsaturation, are consistent with a tetracyclic triterpenoid structure that includes a cyclopropyl group, characteristic of the cycloartane-type triterpenoid.

The ^{1}H, ^{13}C and HMQC NMR spectra implied the presence of a cyclopropyl (δ_H 0.37 (1H, s), 0.58 (1H, s); δ_C 30.1), one hydroxyl group at δ_H 3.78 (1H, dd, 17.4 and 12.6 Hz); δ_C 77.1 and another one at δ_H 3.76 (1H, dd, 17.4 and 12.6 Hz), and δ_H 3.51 (1H, d, 10.5 Hz); δ_C 71.2. The carbon and proton shifts of the basic skeleton of compound **1**, including rings A, B, C, and D, and the cyclopropyl group (C-9, C-10, and C-19), closely resemble the fundamental structure of cycloartane-type triterpenoid [14, 15].

The selected ^{1}H-^{1}H COSY spectrum of compound **1** (Figures 3 and 5) showed correlations in H_1-H_2-H_3, H_5-H_6-H_7, H_{11}-H_{12}, H_{15}-H_{16}-H_{17}-H_{20}, H_{20}-H_{21}, H_{20}-H_{22}-H_{23}, and H_{26}-H_{25}-H_{27}, thereby supporting the presence of the tetracyclic ring system (rings A, B, C, and D) and the side chain structure of compound **1**.

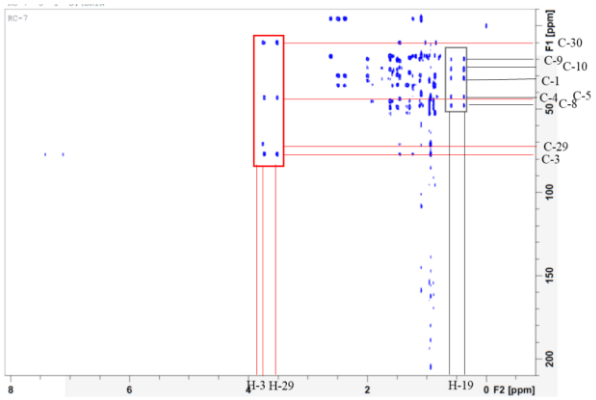

Figure 4. HMBC NMR spectrum of compound **1**

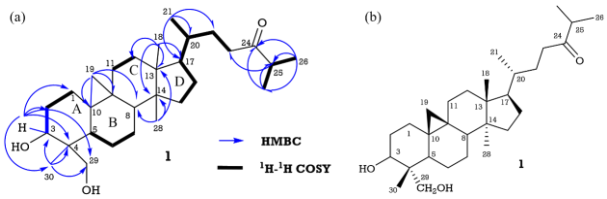

Figure 5. (a) selected HMBC and ^{1}H-^{1}H COSY correlations and (b) structure for compound **1** (cycloartan-3β,29-diol-24-one)

The position of the functional groups in compound **1** was deduced from the HMBC spectra. Correlations were observed between H-18 (δ_H 0.88 (3H, s) to C-12 (δ_C 35.7), two quaternary Csp^3 C-13 (δ_C 45.4) and C-14 (δ_C 48.9), C-17 (δ_C 52.4), and C-28 (δ_C 19.4). These correlations indicate that CH_3-18 is embedded at C-13. Additionally, HMBC cross-peaks were observed from H-28 (δ_H 0.95) to C-8 (δ_C 48.0), and two quaternary Csp^3 C-14 (δ_C 48.9) and C-13 (δ_C 45.4), suggesting that CH_3-28 is embedded at C-14. The methyl protons at δ_H 0.93 (3H, s, H-30) correlate to the methine at δ_C 77.1 (C-3), a quaternary Csp^3 at δ_C 43.8 (C- 4), and oxygenated methylene at δ_C 71.2 (C-29), suggesting that CH_3-30 is embedded at C-4. The methyl at δ_H 0.85 (H-21) correlates to the methine at δ_C 52.4 (C- 17), δ_C 35.9 (C-20), and the methylene carbon at δ_C 31.8 (C-22). The HMBC correlations from the tertiary and secondary methyl protons to their neighboring carbons, as shown in Figures 4 and 5, enabled the assignment of the three tertiary methyl groups to C-4, C-13, and C-14, and the three secondary methyl groups at C-20 and C-25 (2×), respectively.

In the HMBC spectrum, correlations between H-19 at δ_H 0.37 and 0.58 with C-1 (δ_C 30.2), C-5 (δ_C 42.6), C-8 (δ_C 48.0), C-9 (δ_C 20.0), and C-10 (δ_C 25.5) indicate the presence of a cyclopropane ring. Furthermore, the presence of a ketone group at C-24 is evidenced by HMBC correlations from the methyl at δ_H 1.08 (H-26) and δ_H 1.07 (H-27) to ketone carbon group at δ_C 215.7 (C-24). The HMBC correlation of the methine proton at δ_H 3.78 (C-3) to δ_C 43.8 (C-4), δ_C 30.2 (C-2), δ_C 71.2 (C-29), and δ_C 10.3 (C-30) further supports the structure. Moreover, correlations of δ_H 3.76 and 3.51 (CH_2-29) to δ_C 43.8 (C-4) indicate the presence of hydroxyl groups embedded at C- 3 and C-29.

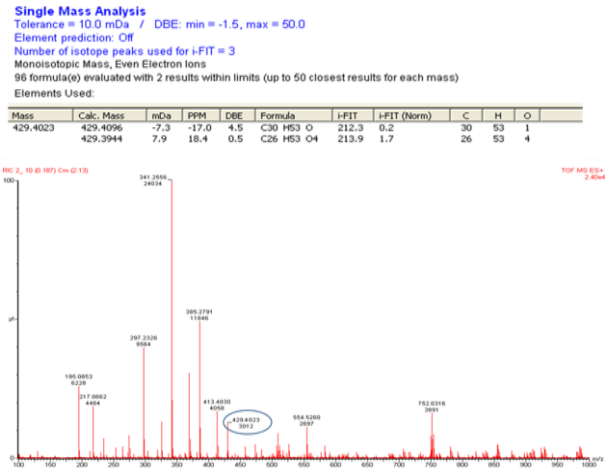

Figure 6. HRMS spectrum of compound **2**

Table 1. NMR data for compound **1**

	Compound **1** (700 and 150 MHz, for ^{1}H and ^{13}C respectively) in $CDCl_3$)				Cycloartan-3β,29-diol-24-one (400 and 100.5 MHz, for ^{1}H and ^{13}C respectively) in $CDCl_3$	
No.	δ_C ppm (mult)	δ_H ppm (Integral, mult, J=Hz)	DEPT-135	HMBC (^{1}H NMR ↔ ^{13}C NMR)	δ_C	δ_H (Integral, mult, J=Hz)
1	30.2 (t)	1.42-1.49 (1H, m) 1.31-1.38 (1H, m)	CH_2	C-2, C-10, C-19	31.7	1.49 (1H, t)
2	30.2 (t)	1.53-1.59 (1H, m) 1.27-1.30 (1H, d, 5.9)	CH_2	C-1, C-10	30.2	1.72 (1H, m)
3	77.1 (d)	3.78 (1H, dd, 16.9, 11.8)	CH-OH	C-1, C-2, C-4, C-29, C-30	77.0	3.75 (1H, dd; 10.5; 4.5)
4	43.8 (s)	-	C	-	43.7	-
5	42.6 (d)	2.46-2.50 (1H, d, 6.9)	CH	C-1, C10	42.5	
6	21.1 (t)	0.79-0.83 (1H, m) 1.20-1.24 (1H, m)	CH_2	C-5, C-7, C-10, C-28	21.0	1.52 (1H, m)
7	28.2 (t)	1.88-1.94 (1H, m) 1.31-1.38 (1H, m)	CH_2	C-5	28.1	1.52 (1H, m)
8	48.0 (d)	1.53-1.59 (1H, m)	CH	C-7, C-12, C-13, C-15, C-18	47.9	
9	20.0 (s)	-	C	-	19.9	
10	25.5 (s)	-	C	-	25.7	
11	25.9 (t)	1.00-1.06 (1H, d, 7.2) 1.20-1.24 (1H, m)	CH_2	C-8, C-12, C-18	25.4	1.49 (1H, t)
12	35.7 (t)	1.72-1.78 (1H, m) 1.27-1.30 (1H, d, 5.9)	CH_2	C-11, C-14, C-17	35.6	1.49 (1H, t)
13	45.4 (s)	-	C	-	45.3	-
14	48.9 (s)	-	C	-	48.8	-
15	33.0 (t)	1.72-1.78 (1H, m) 1.42-1.49 (1H, m)	CH_2	C-12, C-13, C-16	32.9	1.55 (1H, t)
16	26.5 (t)	1.96-2.01 (1H, dt, 17.2, 8.5, 10.5) 1.20-1.24 (1H, m)	CH_2	C-14, C-15	26.4	1.60 (1H, m)
17	52.4 (d)	1.74-1.78 (1H, m)	CH	C-13, C-18	52.3	
18	18.4 (q)	0.88 (3H, s)	CH_3	C-12, C-13, C-14, C-17, C-28	18.0	0.89 (3H, s)
19	30.1 (t)	0.37 (1H, s); 0.58 (1H, s)	CH_2	C-1, C-5, C-8, C-9, C-10	30.0	0.38 (1H, d; 4.2) 0.59 (1H, d; 4.2)
20	35.9 (d)	1.42-1.49 (1H, m)	CH	C-22, C-23	35.9	
21	18.5 (q)	0.85 (3H, d, 6.9)	CH_3	C-17, C-20, C-22	18.4	0.86 (3H, d; 6.6)
22	31.8 (t)	1.60-1.64 (2H, d, 16.8)	CH_2	C-17, C-20, C-21, C-23	32.9	
23	37.7 (t)	2.46-2.50 (1H, d, 6.9) 2.35-2.40 (1H, m)	CH_2	C-20, C-22	37.6	
24	215.7 (s)	-	C=O	-	215.5	
25	41.0 (d)	2.66 (1H, m)	CH	C-23, C-26, C-27	40.9	2.61 (1H, sept; 6.8)
26	18.2 (q)	1.08 (3H, d, 7.1)	CH_3	C-24, C-25, C-26, C-27	18.3	1.09 (3H, d; 6.8)
27	18.2 (q)	1.07 (3H, d, 7.1)	CH_3	C-24, C-25, C-26, C-27	18.1	1.09 (3H, d; 6.8)
28	19.4 (q)	0.95 (3H, s)	CH_3	C-8, C-13, C-14	19.3	0.96 (3H, s)
29	71.2 (t)	3.76 (H, dd, 16.9, 11.8) 3.51 (1H, d; 10.4)	CH_2-OH	C-3, C-4, C-5, C-30	71.1	3.73 (1H, dd; 10.7) 3.52 (1H, d; 10.7)
30	10.3 (q)	0.93 (3H, s)	CH_3	C-3, C-4, C-10, C-29	10.1	0.93 (3H, s)

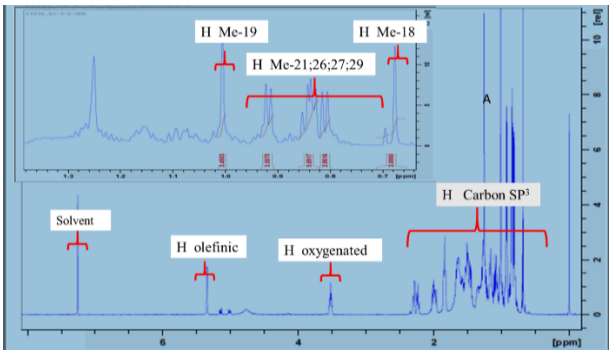

Figure 7. ^{1}H NMR spectrum of compound **2**

The chemical shifts in the ^{1}H, ^{13}C, and other NMR spectra of compound 1, as presented in Table 1, exhibit a strong resemblance to those of the reference compound previously identified by Inada from the isolation of *Aglaia harmsiana* Perkins (Meliaceae) leaves [16]. These spectral features, together with the data obtained from 1D and 2D NMR analyses, suggest that compound **1** is closely related to cycloartan-3β,29-diol-24-one, as depicted in Figure 5.

Based on a comprehensive literature review, compound **1** has only been identified in two *Aglaia* species and has not been found in other plants. This discovery is the second discovery after the first was successfully isolated by Inada *et al.* [16]. Through the interpretation of ^{1}H and ^{13}C-NMR data and considering biogenetic factors, the secondary and primary hydroxyl groups of compound **1** from *Aglaia harmsiana* Perkins (Meliaceae) leaves were located at C-3β and C-29 or C-30, respectively [16].

To establish the position of the primary hydroxyl at C-29 or C-30, a comparison of the carbon resonances of compound **1** with those of cycloartene-3β,29-diol [17] and cycloartane-3β,30-diol was conducted [18]. This comparison revealed that the C-29 and C-30 resonances of compound **1** (δ 71.1 and 10.1, respectively), as well as the ring-A and -B carbon resonances, closely matched those of cycloartene-3β-29-diol (δ 70.5 and 10.2) and differed from those of cycloartane-3β,30-diol (δ 63.1 and 22.0). Consequently, the hydroxymethylene group in compound **1** was assigned to C-29 in an α-equatorial orientation. The ^{13}C NMR chemical shifts of compound **1** from *A.shawiana* Merr. at C-29 and C-30 were 71.2 ppm and 10.3 ppm, respectively. These resonances are very similar to those of compound **1** previously discovered by Inada, confirming that the structure of compound **1** is as shown in Figure 5.

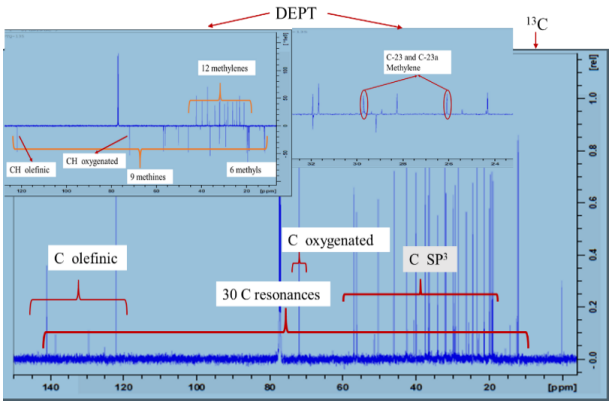

Figure 8. ^{13}C and DEPT-135 NMR spectrum of compound **2**

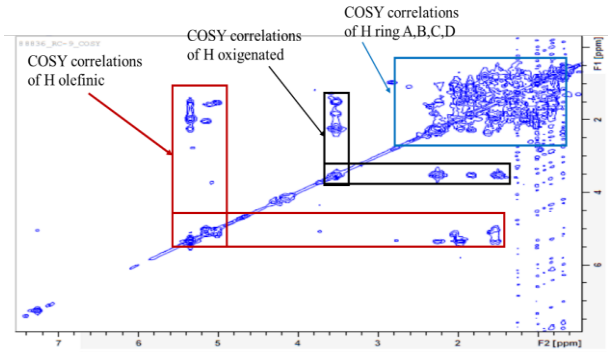

Figure 9. ^{1}H-^{1}H COSY NMR spectrum of compound **2**

In the mass spectrum, compound **1**, isolated by Inada *et al.* [16], exhibited EIMS and HREIMS with an m/z of 458.3768 ([M]$^{+}$, calculated for $C_{30}H_{50}O_3$ 458.3760, 4), 440 (15), 422 (76), 407 (431), 331 (4), 313 (12), and 302.2601. Significant fragments appeared at m/z 331, 313, 302, 127, and 71, which are characteristic fragmentation patterns of 9,19-cycloartane-type triterpenes with one carbonyl in the side-chain and two hydroxyls in the A and B rings. Further, the prominent fragment at m/z 71.0506 (C_4H_7O, base peak) was ascribable to the ion [(Me)$_2$CHC=O]$^{+}$, indicating the presence of a carbonyl function at C-24. Based on this evidence, compound **1** is a cycloartan-24-one derivative bearing one primary hydroxyl group and one secondary hydroxyl group in the A and B rings.

The ^{1}H NMR spectrum of compound **1** from *Aglaia harmsiana*, analyzed by Inada in 1995 with the aid of 2D NMR studies (COSY and NOESY), indicated the presence of three tertiary methyl groups (δ 0.89, 0.94, and 0.96), three secondary methyl groups at δ 0.86 (d, J = 6.6Hz) and δ 1.09 (6H, d, J=6.8 Hz). Additionally, doublets at δ 0.38 (J=4.2 Hz) and 0.59 (J = 4.2 Hz) were observed, characteristic of the non-equivalent protons of a cyclopropyl methylene group.

Meanwhile, the ^{1}H NMR spectrum of compound **1** from *Aglaia shawiana* Merr. (Table 1) indicated the presence of three tertiary methyl groups δ 0.88 (H-18), 0.93 (H-30), and 0.95 (H-28), three secondary methyl groups at δ 0.85 (d, J = 6.9 Hz), δ 1.07 (3H, d, J=7.1 Hz) and δ 1.08 (3H, d, 7.1 Hz). Additionally, doublets at (δ 0.37 (s) and 0.58 (s)) were observed, characteristic of non-equivalent protons of a cyclopropyl methylene group. Based on the strong similarity of the obtained NMR spectral data, despite slight differences likely attributable to variations in NMR frequency, it can be concluded that compound 1 from *Aglaia shawiana* leaves is the same as that previously discovered by Inada *et al.* [16].

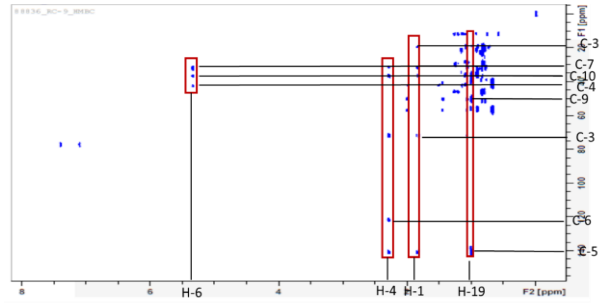

Figure 10. HMBC NMR spectrum of compound **2**

Table 2. NMR data for compound **2**

Compound **2** (700 and 150 MHz, for ^{1}H and ^{13}C respectively) in $CDCl_3$					23a-Homostigmast-5en-3β-ol (400 and 100.5 MHz, for ^{1}H and ^{13}C respectively) in $CDCl_3$ [19]	
No.	δ_C (ppm) (mult.)	δ_H (ppm) (ΣH, m, *J* Hz)	DEPT 135	HMBC (^{1}H NMR $\leftrightarrow$ ^{13}C NMR)	δ_C (ppm)	δ_H (ppm) (ΣH, m, *J* Hz)
1	37.4 (t)	1.85 (1H, m) 1.83 (1H,m)	CH_2	C-3, C-5, C-10	37.3	1.86 (1H, m) 1.81 (1H, m)
2	32.1 (t)	1.54 (2H, m)	CH_2		31.9	1.52 (2H, m)
3	72.0(d)	3.52 (1H, sept, 3.6)	CH-OH		71.8	3.50 (1H, sept)
4	42.4 (t)	2.26 (2H, m)	CH_2	C-3, C-5, C-6, C-10	42.3	2.25 (2H, m)
5	141.0 (s)	-	C		140.8	-
6	121.9 (d)	5.35 (1H, br d, 6,8)	CH	C-4, C7, C-10	122.1	5.34 (1H, brd, 4.8)
7	31.9 (t)	1.93 (1H, m) 1.84 (1H, m)	CH_2	C-5, C-9	31.7	1.93 (1H, m); 1.84 (1H,m)
8	31.9 (d)	1.96-2.00 (1H, m)	CH		31.9	1.98 (1H, m)
9	50.3 (d)	0.97-0.98 (1H, m, 11.9)	CH		50.2	0.97 (1H, m)
10	36.7 (s)	-	C		36.5	-
11	21.2 (t)	1.49 (2H, m)	CH_2	C-13	21.1	1.46 (2H, m)
12	40.0 (t)	2.02 (1H, m); 1.14 (1H, m)	CH_2	C-13, C-14, C-17, C-18	39.8	2.04 (2H, m)
13	42.5 (s)	-	C		42.3	-
14	56.9 (d)	1.06 (1H, m)	CH	C-9, C-14	56.9	1.06 (1H, m)
15	24.5 (t)	1.50 (1H, m)	CH_2	C-13	24.4	1.54 (1H, m)
16	28.4 (t)	1.19 (2H, m)	CH_2		28.2	1.16 (2H, m)
17	56.2 (d)	1.06-1.11 (1H, m)	CH	C-18	55.0	1.10 (1H, m)
18	12.0 (q)	0.68 (3H, s)	CH_3	C-12, C-13, C-17	12.0	0.75 (3H, s)
19	19.6 (q)	0.99 (3H, s)	CH_3	C-5, C-9, C-10	19.4	0.80 (3H, s)
20	36.3 (d)	1.46-1,50 (1H, m)	CH	C-17	36.2	1.43 (1H, m)
21	18.9 (q)	1.00 (3H, d, 8,0)	CH_3	C-17, C-20, C-22	18.8	0.99 (3H, d, 8.0)
22	34.1 (t)	1.50 (1H, m) 1.22 (1H, m)	CH_2		34.0	1.51 (1H,m); 0.99(1H,m)
23	29.9 (t)	1.26 (2H, m)	CH_2	C-23a, C-24	29.7	1.52 (2H, m)
23a	26.2 (t)	1.17 (2H, m)	CH_2		26.1	1.12 (2H, m)
24	46.0 (d)	0.93-0.95 (1H, m, 5.6)	CH		45.9	0.91(1H,m)
25	29.3 (d)	1.83 (1H, m)	CH	C-24	29.3	1.81(1H,m)
26	19.2 (q)	0.80 (3H, d, 6,9)	CH_3	C-24, C-25, C-27	19.0	0.79 (3H, d, *J*=6.8)
27	20.0 (q)	0.83 (3H, d, 6,8)	CH_3	C-24, C-25, C-26	19.8	0.83 (3H, d, *J*= 6.8)
28	23.2 (t)	1.25 (1H, m) 1.20 (1H, m)	CH_2	C-24, C-29, C-23a	23.1	1.23 (2H, m)
29	12.1 (q)	0.83 (3H, t, 7,0)	CH_3	C-24, C-28	12.1	0.86 (3H, t, *J*=7.0)

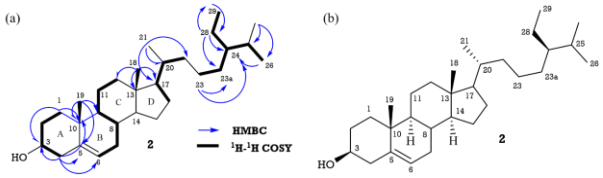

Figure 11. Selected HMBC and ^{1}H-^{1}H COSY correlations and structure for compound **2**

3.2. Compound 2

Compound **2** was obtained as a white amorphous powder. Its molecular formula, $C_{30}H_{52}O$, was established from the HR-TOFMS, which showed m/z 429.4023 $[M + H]^+$ (calculated for $C_{30}H_{53}O$, m/z 429.4096), as shown in Figure 6 and supported by NMR data. This composition required five degrees of unsaturation. The ^{1}H NMR ($CDCl_3$, 700 MHz, ppm) spectrum of compound **2** displayed two tertiary methyl groups at δ_H 0.68 (3H, *s*, Me-18) and 1.25 (3H, *s*, Me-19), three secondary methyl groups at δ_H 0.84 (3H, d, *J* = 8.0 Hz, Me-21), 0.82 (3H, d, *J* = 6.9 Hz, Me-26) and 0.83 (3H, d, *J* = 6.8 Hz, Me-27), and one primary methyl at δ_H 0.92 (3H, t, *J* = 7.0 Hz, Me-29). Additionally, one olefinic and oxygenated methine (CH-OH) signals were also observed in ^{1}H-NMR at δ_H 5.35 (1H; br. d; *J* = 6.9 Hz; H-6) and δ_H 3.50 (1H; m; *J* = 10.0, 6.0, 3.6 Hz, H-3), respectively, as shown in Table 2 and Figure 7.

The ^{13}C NMR ($CDCl_3$, 175 MHz, ppm) spectrum showed 30 carbon resonances, which were classified by their chemical shifts, DEPT, and HMQC spectra as follows: six methyl groups (two tertiary at δ_C 12.0 and 19.6, three secondary at δ_C 18.9, 19.2, 20.0, one primary at 12.1), twelve methylene groups at δ_C 37.4, 32.1, 42.4, 31.9, 21.2, 40.2, 24.5, 28.4, 34.1, 29.9, 26.2 and 23.2, nine methine groups at δ_C 32.0, 50,3, 56.9, 56.2, 36.3, 46.0, 29.3 (one oxygenated at δ_C 72.0 and one olefinic carbons at 121.9), and three quaternary carbons at δ_C 36.7, 42.5 (1 olefinic at δ_C 141.0).

Moreover, this olefinic functionality accounted for 1 out of the total 5 hydrogen deficiency index. The remaining four hydrogen deficiency indexes were consistent with the tetracyclic structure. These are characteristic resonances of sterol, with one double bond like *β*-sitosterol or stigmas-5-en-3*β*-ol previously published, but the addition of 1 methylene, which resonates at 26.2 in compound **2** that can be seen clearly in the ^{13}C and DEPT spectra (Figure 8), which was not found in sitosterol [20]. The selected ^{1}H-^{1}H COSY spectrum of **2** (Figures 9 and 11) showed correlations in H_1-H_2-H_3-H_4, H_6-H_7-H_8-H_9-H_{10}-H_{11}-H_{12}, H_{16}-H_{17}-H_{20}-H_{22}, H_{23}-H_{23a}-H_{24}-H_{28}-H_{29}, H_{24}-H_{25}-H_{26}, those correlations indicated a tetracyclic structure.

The position of the functional group in compound **2** was determined from the HMBC spectra. In the HMBC spectrum, correlations were observed between H-19 (CH_3-19 δ_H 1.00) and a quaternary Csp^2 C-5 (δ_C 141.0), C-9 (δ_C 50.3), and C-10 (δ_C 36.7), indicating that CH_3-19 was embedded at C-10. Additionally, HMBC cross-peaks were observed from H-18 (CH_3-18 δ_H 0.68) to C-12 (δ_C 40.0), C- 17 (δ_C 56.2), and C-13 (δ_C 42.5), suggesting that CH_3-18 was embedded at C-13. The methyl protons at δ_H 0.91 (H- 21) showed correlations with the methine at δ_C 56.2 (C-17), δ_C 36.3 (C-20), and the carbon at δ_C 34.1 (C-22). The HMBC spectrum displayed correlations between the methyl protons at δ_H 0.83 (H-29) and δ_C 46.0 (C-24), as well as δ_C 23.2 (C-28).

Correlations were observed between δ_H 0.83 (H-27) and the methine at δ_C 46.0 (C-24), δ_C 29.3 (C-25), and the methyl group at δ_C 19.2 (C-26). Furthermore, correlations were noted between δ_H 0.80 (H-26) and the methine at δ_C 46.0 (C-24), δ_C 29.3 (C-25), and the methyl at δ_C 20.0 (C- 27). These HMBC correlations from the tertiary, secondary, and primary methyl protons to their neighbor carbons (Figures 10 and 11) enabled the assignment of the two tertiary methyl groups at C-10 and C-13, secondary methyl groups at C-20 and C-25 (2×), and the primary methyl at C-29, respectively.

Furthermore, the olefinic proton at δ_H 5.35 (1H, br d, *J* = 6.9 Hz, H-6) was correlated to methylene carbons at C-4 (δ_C 42.4) and C-7 (δ_C 31.9), as well as with the quaternary carbon at C-10 (δ_C 36.7). Correlations were also observed between the methylene protons H-4 (δ_H 2.26, 2H, m) and C-5 (δ_C 141.0), C-6 (δ_C 121.9), C- 3(δ_C 72.0), and C-10 (δ_C 36.7). Additionally, the proton H-1 (δ_H 1.85, 1H, m) correlated with C-3 (δ_C 72.0) and C-5 (δ_C 141.0), while another proton H-1 (δ_H 1.83, 1H, m) correlated with C-10 (δ_C 36.7), C-3 (δ_C 72.0), and C-5 (δ_C 141.0). These HMBC correlations enabled the assignment of an olefinic moiety at C-5 and C-6 ($\Delta^{5,6}$) and a hydroxyl group located on C-3.

The HMBC spectrum of **2** exhibited interactions of methylene protons at δ_H 1.26 and 1.20 (H-28) with the methine at δ_C 46.0 (C-24), the carbon methyl δ_C 12.1 (C- 29), and the methylene at δ_C 26.2 (C-23a). Correlations were also observed between methylene protons H-23 (δ_H 1.26, 2H, m) and the methylene carbons at δ_C 26.2 (C- 23a) and δ_C 46.0 (C-24). These HMBC correlations, along with the ^{13}C NMR, DEPT, and COSY spectra, defined the presence of sp^3 methylene carbon at δ_C 29.9 (C-23) and δ_C 26.2 (C-23a). Comparison of the ^{1}H NMR and ^{13}C NMR spectral data with *β*-sitosterol suggested the presence of Δ^5 steroid-type structure similar to stigmas-5-en-3*β*-ol, which has one double bond as previously reported [21]. The similar signals, except for a marked difference with the addition of one methylene carbon at C-23, led to the identification of C-23a, named C-23a-Homo after C-23 (δ_C 26.2, δ_H 1.17, (2H, m, H-23a)). Consequently, compound **2** is named 23a-Homostigmast-5-en-3*β*-ol, with its two-dimensional structure (Figure 11).

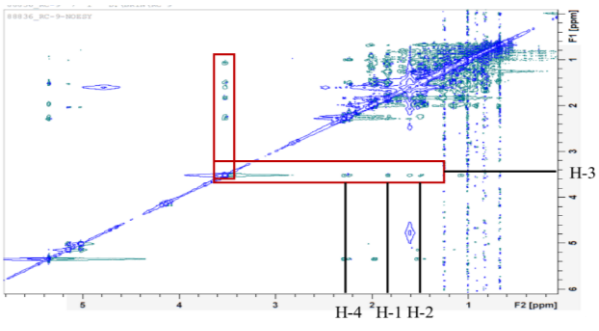

Figure 12. NOESY NMR spectrum of compound **2**

The relative configuration of compound **2** was identified by a NOESY experiment (Figure 12), which showed the NOESY correlations between H-3 (δ_H 3.52) and H-4 (δ_H 2.261), H-1 (δ_H 1.83), and H-2 (δ_H 1.54). In the NOESY spectrum of compound **2**, no correlation was found between H-18β (Me-18β at δ_H 0.68) or H-19β (Me-19β at δ_H 0.99) and H-3, H-4, H-1, and H-2. This absence of correlations indicates that the hydroxyl group at C-3 is β-oriented. A detailed comparison of the ^{1}H NMR and ^{13}C NMR spectral data of compound **2** with those of 23a-homostigmast-5-en-3β-ol (Table 2), previously isolated from the *n*-hexane fraction of the roots of *Fumaria parviflora*, confirmed the structure of compound **2** as shown in Figure 11 [19].

The spectrum of compound **2** shows the presence of additional olefinic proton signals, likely impurities, at δ_H 5.0-5.2 ppm. In the ^{13}C-NMR spectra, two other olefinic carbons were observed at δ_C 129 and 138 ppm. However, these signals showed no correlations with other signals in the HMBC spectrum. The ^{13}C signal at δ_C 141.0 in the DEPT spectrum resembles that of a methylene group. However, it does not correlate with signals in the ^{1}H, HSQC, and HMBC spectra, suggesting that this signal represents a quaternary carbon. These signals are, therefore, presumed to be impurities present in compound **2**.

4. Conclusion

A cycloartane-type triterpenoid, specifically cycloartan-3β,29-diol-24-one (**1**), was isolated from the *n*-hexane leaf extract of *Aglaia shawiana*, a member of the Meliaceae family. Until now, this cycloartane compound has only been identified in two species of *Aglaia*. It was first isolated from *A. harmsiana* by Inada in 1995, and the study's isolation from *Aglaia shawiana* Merr marks the second successful identification of this compound. Additionally, another compound, 23a-homostigmast-5-en-3β-ol (**2**), a steroid with a unique side chain, has also been isolated from this species.

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