MOLEKUL
eISSN: 2503-0310

Articles
https://doi.org/10.20884/1.jm.2023.18.2.6205

Cholestan Steroids from The Stem Bark of *Aglaia angustifolia* Miq and Their Cytotoxic Activity against MCF-7 Breast Cancer Cell Lines.

Ricson Pemimpin Hutagaol[1*], Tjandrawati Mozef[2], Sofa Fajriah[2], Gian Primahana[2], Unang Supratman[3,4], Desi Harneti[3], Ace Tatang Hidayat[3,4], Khalijah Awang[5], Yoshihito Shiono [6]

[1.]Department of Chemistry, Faculty of Mathematics and Natural sciences, Nusa Bangsa University, Bogor 16166, Indonesia.
[2.]Research Center for Pharmaceutical Ingredients and Traditional Medicine, National Research and Innovation Agency (BRIN), Serpong Tangerang Selatan 15314, Indonesia.
[3.]Department of Chemistry, Faculty of Mathematics and Natural sciences, Universitas Padjadjaran, Jatinangor 45363, Indonesia.
[4.]Central Laboratory of Universitas Padjadjaran, Jatinangor 45363, Indonesia
[5.]Department of Chemistry, Faculty of Sciences, University of Malaya, Kuala Lumpur 59100, Malaysia.
[6.]Department of Food, Life and Environment Science, Faculty of Agriculture, Yamagata University, Yamagata,997-8555, Japan.

*Corresponding author email: ricsonpemimpin70@gmail.com

Received June 23, 2022; **Accepted** April 14, 2023; **Available online** July 20, 2023

ABSTRACT. With about 120 species, Aglaia is one of the largest genera of the plant family Meliaceae (the mahogany plants). Various Aglaia species have been investigated since the 1960s for their phytochemical constituents and biological properties. This research objective was to find secondary metabolites that have activity as anti-breast cancer compounds from endemic Indonesian Aglaia, such as *Aglaia angustifolia* Miq. Two cholestan type steroids, stigmast-5en-3α-acetat (**1**), as a new steroid with α-sterochemistry of acetyl moiety at C-3 and 23a-homostigmast-5en-3β-ol (**2**), with unusual side chain were isolated for the first time from the stem bark of *Aglaia angustifolia* Miq or known as segara tree in Kalimantan. The chemical structures of two steroids were identified with spectroscopic data, including IR, NMR (1H, 13C, DEPT 135°, HMQC, HMBC, NOESY, ^{1}H-^{1}H COSY) and HRTOF-MS, as well as by comparing with previously reported spectral data. These two steroids were isolated for the first time from this genus. Steroids **1** and **2** were evaluated for cytotoxic activity against MCF-7 breast cancer cells and showed weak activity with IC_{50} values of 829.0 and 903.0 μg/mL, respectively.

Keywords: *Aglaia angustifolia*, cholestan steroids, cytotoxic activity

INTRODUCTION

Steroids are a structurally diverse group of natural products that exist in a variety of organisms exhibiting extensive biological activities (Li et al., 2021; Ratnaweera et al., 2015; Kikuchi et al., 2017; Gu et al., 2018; Ren et al., 2019). To date, more than 100 steroidal agents have been approved as pharmaceuticals by the FDA for the treatment of cancer, heart failure, inflammation, pain, traumatic brain injury etc (Kim et al., 2018). Continuous efforts in searching for structurally novel and active steroids may thus provide structural lead for drug development, which will also make the prosperity of the steroid chemistry. *Aglaia lour.,* the largest genus of subtropical and tropical angiosperm family Meliaceae (the mahagony plants), consists of 130 species. Aglaia is native to the tropical rain forests of the Indo-Australian region, ranging from India and Srilanka eastward to Polynesia and Micronesia. Various *Aglaia* species have been investigated since 1960s for their phytochemical constituents and biological proterties (Agarwal et al., 2021; Farabi et al., 2018). *Aglaia angustifolia* mainly distributed in Indonesia (Sumatera, Kalimantan) and declared almost extinct, until today only a few phytochemical studies have been carried out to identify its active metabolites (Hutagaol et al., 2021); Hutagaol et al., 2020). Previous phytochemical studies on some species *Aglaia* plants reported the presence of a series of secondary metabolite such as triterpenoids, lignans, rocaglate derivatives, sesquiterpenoids, tetraterpenoids and steroids. Phytochemical research of the species *A. angustifolia* has not been widely carried out, steroids that have been found and published previously from this species are stigma-4-en-3on (Hutagaol et al., 2022). Previous phytochemical studies on some species Aglaia plants reported the presence of a series of secondary metabolite such as triterpenoids, lignans, rocaglate

derivatives, sesquiterpenoids, tetraterpenoids and steroids. Those which have been shown to posses antifungal, insecticidal and anticancer properties (Zhang et al., 2012; Awang et al., 2012; Harneti et al., 2014; Harneti & Supratman, 2021).

Fractionation of the *n*-hexane and ethyl acetate extracts from the stembark of this *Aglaia angustifolia* led to the isolation of one steroid compound **1**, as a new steroid with *α*-sterochemistry of acetyl moety at C-3 along with one knew steroid compound **2**, with unusual side chain (**Figure 1**). Based on the literature that the two steroids **1** and **2** from the results of this study are the first steroid structures found in Aglaia. The Isolation, structure elucidation of compounds based on spectroscopic data are described herein. Breast cancer is one of a kind cancer by prevalence highest in the world. According to WHO in 2020, as many as 2.1 million womans has been diagnosed have breast cancer every year. In vitro testing using modeling culture being a method dependable for find a cure for cancer. MCF-7 cells have used for more than 40 years in research breast cancer. Cell culture was isolated from the patient breast cancer which metastasize to pleura. This study used MCF-7 cells because MCF-7 cells are many cells used for in vitro tests and has the best form. MCF-7 cells are included in breast cancer subtype luminal A which has ER+ molecular characteristics, PR+ and HER2- (Lailatul & Nurrachma, 2020). As part of our studies on anti breast cancer cells candidate compounds from Indonesia *Aglaia* plants. The obtained steroid compounds were evaluated biological activity against MCF-7 human breast cancer cell line and showed weak activity with IC_{50} 829.0 *μ*g/mL (**1**) and 903.0 *μ*g/mL (**2**), with cisplatin as standard 38.1 *μ*g/mL. The biological activity test method used in this study used Presto Blue method with cisplatin as a standard compound, but different in the compounds tested, which in this study were tested against steroids **1** and **2**, while tested limonoids (Supriatno et al., 2018).

EXPERIMENTAL SECTION

General Experimental Procedure

Optical rotations were measured on a Perkin Elmer 341 Polarimeter (Waltham, MA, USA). The IR Spectra were recorded on a Perkin Elmer 1760 X FT-IR in KBr (Waltham, MA, USA). Mass spectra were obtained with a Water Qtof. HR-MS XEV^{otm} mass spectrometer (Waters, Milford, MA, USA). 1D NMR spectra (^{1}H, ^{13}C and DEPT) and 2D spectra (COSY, HSQC, HMBC and NOESY) spectra were recorded with a Bruker 600 MHz/Topspin 3.5P17 spectrometer for **1** and JEOL JNM ECZ-600 spectrometer for **2**, (at 600 MHz for ^{1}H and 125 MHz for ^{13}C-NMR, with $CDCl_3$ as a solvent, chemical shift were given on a *δ* (ppm) scale and both using tetra methyl silane (TMS) as the internal standard. Technical solvents were distilled prior to use for maceration, isolation and spectral grade solvents were employed for spectroscopic measurements. Chromatographic separation were carried out on silica gel 60 (70-230 mesh and 230-400 mesh; Merck, Darmstadt, Germany) and Octa Dodecyl Silane (ODS Fuji Sylisia, Japan). TLC plates were precoated with silica gel GF_{254} (Merck, 0.25 mm). Spots were visualized under UV light of 254 nm and 365 nm simultaneously and by spraying with 10% H_2SO_4 in ethanol or vanillin reagent followed by heating.

Plant Material

The stem bark of *A. angustifolia* (Miq.) was collected from Bogor Botanical Garden, West Java Indonesia on February 2017. The following is the taxonomy of the species *A. angustifolia* (Miq.), kingdom : Plantae, subkingdom : Tracheobionta, superdivision : Spermatophyta, division : Mangnoliophyta, class : Mangnoliopsida, subclass : Rosidae, order: Sapindales, family : Meliaceae, genus: Aglaia, species : *A.angustifolia* (Miq.). The plant was identified by Center for Plant Conservation Botanic Gardens Bogor, Indonesia, and a voucher specimen (II.K.57a) deposited at Herbarium.

Extraction and Isolation

Dried powder stem bark (1.97 kg) of *A. angustifolia* were extracted exhaustively with *n*-hexane at room temperature (3 x 24 hours, each 3 L) filtered, solution evaporated to give 25 g *n*-hexane extracts. Powder stem bark of *n*-hexane maceration residue dried and than extracted exhaustively with EtOAc at room temperature (3 x 24 hours, each 3 L) filtered, to give solution evaporated to give 19.7 extracts. The EtOAc extract of *A.* angustifolia (19 g) was subjected to vacum liquid column chromatography (VLC) packed with silica gel by gradient elution of *n*-hexane - EtOAc - MeOH (100:0:0 - 0:0:100) to give 8 fractions (A - G). Fr. B 1.01 g was subjected to column chromatogaphy (CC) over silica gel G_{60} (70 -230 mesh) using 5% gradient mixture *n*-hexane:CH_2Cl_2:EtOAc (100:0:0 - 0:0:100) to give 8 fractions (B1–B8). Fr. B3 76.3 mg was subjected to CC over silica gel G_{60} (400 mesh) using isocratic mixture *n*-hexane:CH_2Cl_2 (98:2) to give 8 combined fraction (B3a - B3g). Fr. B3d (28.8 mg) was subjected to CC over silica gel (400 mesh) using isocratic mixture *n*-hexane:CH_2Cl_2:EtOAc (98:1.5:0.5) to yield compound **1** (6.9 mg), Rf: 0.65 (*n*-hexane: CH_2Cl_2:EtOAc:MeOH - 1.8:0.15:0.05:0.05). 24 g of *n*-hexane crude was subjected to vacum liquid column chromatography (VLC) over a silica gel using a 10% gradient mixture of *n*-hexane - EtOAc - MeOH as eluent. A total of 7 (A - G) combine fraction were obtained. Fr B (2.58 g) was subjected to CC over silica gel (70 - 230 mesh) using 2% gradient mixture *n*-hexane - EtOAc - MeOH eluent, 20 fr (B1 - B20) were obtained. Fr. B8 (261.2 mg) were subjected to CC over ODS using isocratic mixture MeOH - H_2O = 90:10 eluent. 24.4 mg of combined fr. 66 to fr. 70 purified with prep. TLC using 85:10:5 = *n*-hexane – EtOAc – MeOH eluent to give compound **2** (4.8 mg)

Bioassays for Cytotoxic Activity

The cytotoxicity of the compounds against MCF-7 human breast cancer cell was measured using the PrestoBlue cell viability assay. The cells were maintained in a Roswell Park Memorial Institute (RPMI) medium supplemented with 10 % (v/v) Fetal Bovine Serum (FBS) and 50 µL/50 mL antibiotics. Cultures were incubated at 37^0 C in a humidified atmosphere of 5 % CO_2. Tumor cells were seeded in 96-well microliter plates at 1.7 x 10^4 cells per well. After 24 h, compounds were added to the wells. After 96 h, cell viability was determined by measuring the metabolic conversion of resazurin substrate into pink fluorescent resorufin product resulting from reduction in viable cells. The PrestoBlue assay results were read using multimode reader at 570 nm (reff: 600 nm). All compounds were tested at eight concentration (7.81; 15.63; 31.25; 62.50; 125.00; 250.00; 500.00; 1000.00) µg/mL in 100% DMSO with a final concentration of DMSO of 2.7 % in each well. Each concentration of the compounds was tested in two parallels experiments. IC_{50} values were calculated by linier regression method using Microsoft excel software.

RESULTS AND DISCUSSIONS

Stigmast-5en-3α-acetat (**1**), was obtained as a white amorphous powder, with $[\alpha]_D^{29.4} -1.67^0$(c, 26g, $CHCl_3$). Its molecular composition $C_{31}H_{52}O_2$, was established from the HR-TOFMS found m/z 479.3839 $[M + Na]^+$ (calculated for $C_{31}H_{52}O_2Na$, m/z 479.3865) and NMR data (**Table 1**), thus required six degree of unsaturation. The IR spectra showed the presence of a methine sp^3 – CH st (2956; 2939; 2868; and 2852 cm^{-1}), carbonyl ester – C=O st (1731 cm^{-1}), methine olefinic bend = CH (1464; 1383 and 1372 cm^{-1}), and a ether group – C – O st (1262; 1245 and 1040 cm^{-1}).

The ^{1}H NMR ($CDCl_3$, 600 MHz) spectrum of **1** displayed the presence of two tertiary methyls at [δ_H 0.68 (3H, *s*, Me-18) and 1.01 (3H, *s*, Me-19) pppm], three secondary methyls at [δ_H 0.81 (3H, d, *J*=6.0 Hz, Me-21), 0.81 (3H, d, *J*=6.2 Hz, M e-26) ppm and 1.00 (3H, d, *J*=6.2 Hz, Me-27) ppm] and one primary methyl at δ_H 0.78 (3H, t, *J*=1.9 Hz, Me-29) ppm. The acetyl, olefinic and oxygenated methine signals were also observed in ^{1}H-NMR at δ_H 2.04 (3H, s, Me-1'), 5.37 (1H, d, *J*=6.0 Hz, H-6) and 4.60 (1H, m, H-3) ppm, respectively.

The ^{13}C NMR ($CDCl_3$, 150 MHz) spectrum showed 31 carbon resonances, which were classified by their chemical shifts, DEPT and HMQC spectra as 7 methyls (2 tertiary at δ_C 12.0 and 19.5; 3 secondary at δ_C 19.0, 19.2, 20.0 1 primary at 12.2 and 1 acetyl at 21.6), 11 methylenes at δ_C 37.4, 28.2, 38.3, 32.1, 21.2, 39.9, 24.5, 28.4, 34.1, 26.2, and 23.3 ppm. 9 methines at δ_C 32.1, 50,3, 56.8, 56.2, 36.3, 46.0, 29.3 ppm (1 oxygenated at δ_C 74.2 ppm and 1 olefinic carbons at 122.9) and 4 quaternary carbons at δ_C 36.8, 42.5 ppm (1 olefinic at δ_C 139.9 and 1 carbonyl ester at δ_C 170.8). These functionalities accounted for 2 out of the total 6 hydrogen deficiency index. The remaining four hydrogen deficiency index were consistent with the tetracyclic structure. These are characteristic resonances of sterol, like β- sitosterol or stigmas-5-en-3β-ol previously published, but absence of hydoxyl group at C-3 substituted with acetyl signals at [δ_H 2.04 (3H, s) δ_C 21.6, 170.8] (Martínez et al., 2017)

The selected ^{1}H-^{1}H COSY spectrum of **1** (**Figure 2**), showed correlations in H_2-H_3-H_4, H_6-H_7-H_8-H_9, H_{11}-H_{12}, H_{14}-H_{15}-H_{16}-H_{17}-H_{20}, H_{23}-H_{24}-H_{25}-H_{26}, and H_{24}-H_{28}-H_{29} supporting the presence of tetracyclic structure of **1**.

The functinal group position of **1** was deduced from the HMBC spectra (**Figure 2**). There were correlations between H-19 (δ_H 1.01) to a quaternary C sp^2 C-5 (δ_C 139.9); C-1 (δ_C 37.4); C-9 (δ_C 50.3) and C-10 (δ_C 36.8) these correlations indicating that CH_3-19 embeded at C-10. Some HMBC cross peaks were observed from H-18 (δ_H 0.68) to C-12 (δ_C 39.9); C-17 (δ_C 56.2) and C-13 (δ_C 42.5) these correlations suggesting that CH_3-18 embeded at C-13. The methyl protons at δ_H 0.78 (H-29) correlate to the methine at δ_C 46.0 (C-24) and with carbon δ_C 23.3 (C-28). The methyl at δ_H 0.81 (H-21) correlate to the methine at δ_C 56.2 (C-17) and with carbon δ_C 36.3 (C-20). The HMBC correlations from the tertiary, secondary and primary methyl protons to their neighboring carbons, enabled the assignment of the two tertiary methyls at C-10 and C-13, secondary methyl at C-20 and C-25 (2×) as well as a primary methyl at C-29, respectively.

Furthermore, the olefinic proton, δ_H 5.37 (1H, d, *J* = 6.0 Hz, H-6) was correlated to olefinic carbon at C-5 (δ_C 139.9), methylene carbon at C-4 (δ_C 38.3) and C-7 (δ_C 32.1), quaternary carbon at C-10 (δ_C 36.8) and a methine carbon at C-8 (δ_C 32.1) indicating an olefinic moiety was located at C-5 and C-6 ($\Delta^{5,6}$). Correlations from oxygenated methine proton at H-3 (δ_H 4.60) and methyl acetyl at H-31, C**H**$_3$-COO (δ_H 2.04) to carbonyl ester at C-30, CH_3-**C**OO (δ_C 170.8), were used to assign an acetyl group which was located on C-3.The spectrum of **1** shows the presence of other olefinic proton signals as impurities (δ_H 5.0-5.5 ppm). In the ^{13}C-NMR and DEPT 135 spectra, two other olefinic carbons at δ_C 125-140 ppm were also seen. However on the HMBC spectrum, the above signals have no correlation with other signals, so that those signals were assumed to be impurities found in compound **1**.

The relative configuration of compound **1** was identified by a NOESY experiment (**Figure 3**), which showed the NOESY correlations between H-19β (Me-19β at δ_H 1.01) with H-4 (δ_H 2.31) and H-1 (δ_H 1.87).

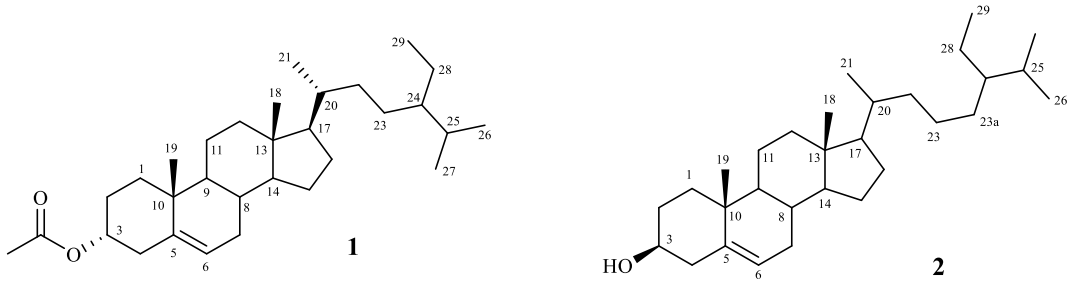

Figure 1. Chemical structure of steroids **1** and **2**

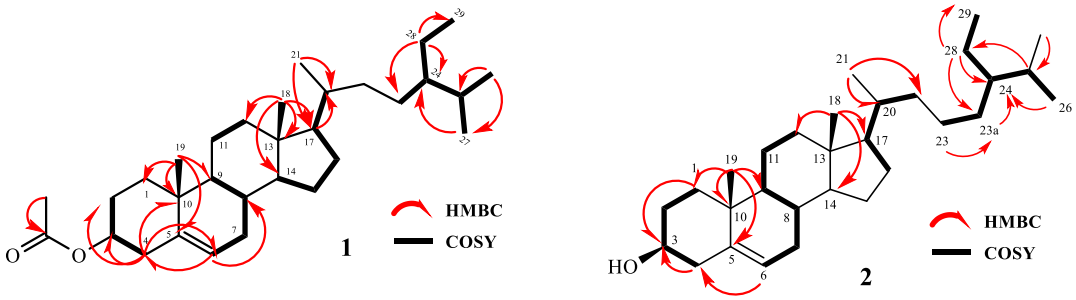

Figure 2. Selected ^{1}H-^{1}H COSY and HMBC for Steroids **1** and **2.**

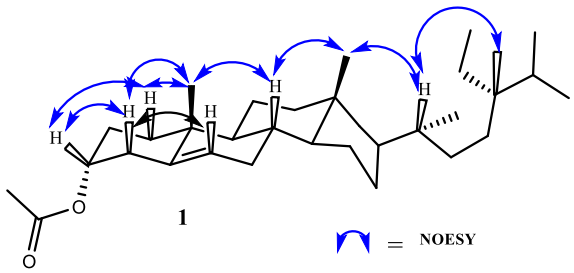

Figure 3. Selected NOESY correlations for steroid **1.**

Correlations also shown by H-3 (δ_H 4.60) H-4 and H-1. These correlations indicated that the acetyl group at C-3 is *α*-oriented. Similar to those observations from NOESY, showed the cross peak between H-18*β* (Me-18*β* at δ_H 0.68) with H-20 (δ_H 1.33), and cross peak between H-19*β* with H-20. Correlation also shown by H-20 with H-24 (δ_H 0.92), these NOESY correlations indicated that H-20 and H-24 was *β*-oriented. Compound **1** was similar to a compound that have been found previously from the stem bark of *Chisocheton cumingianus* (Meliaceae) and from the aerial parts of *Polygonum bellardii*, *β*-sitosterol-3-O-acetate but with difference stereochemistry of acetyl moety at C-3 (Katja et al., 2017); (El-kader et al., 2012). Therefore, compound **1** was elucidated to be a new derivative cholestan-type steroid with *α*-oriented relative configuration of acetyl group at chiral carbon C-3 and was named 24*S*, 24-ethylcholest-5en-3*α*-acetate.

23a-Homostigmast-5en-3β-ol (**2**) was obtained as a white amorphous powder. Its molecular composition $C_{30}H_{53}O$, was established from the HR-TOFMS found m/z 429.4023 $[M + H]^+$ (calculated for $C_{31}H_{52}O_2Na$, m/z 429.4096) and NMR data (**Table 2**), thus required five degree of unsaturation. The IR spectra showed the presence of a methine sp^3 – CH st (2959; 2937; and 2868 cm^{-1}), hydroxyl – O-H st (3424 cm^{-1}), methine olefinic bend = CH (1464; and 1379 cm^{-1}), and a ether group – C – O st (1056 and 1050 cm^{-1}).

The ^{1}H NMR ($CDCl_3$, 600 MHz) spectrum of **2** displayed the presence of two tertiary methyls at [δ_H 0.67 (3H, *s*, Me-18) and 0.99 (3H, *s*, Me-19) ppm], three secondary methyls at [δ_H 0.90 (3H, d, *J* = 8.0 Hz, Me-21), 0.80 (3H, d, *J* = 6.8 Hz, Me-26) and 0.83 (3H, d, *J* = 6.8 Hz, Me-27) ppm] and one primary methyl at δ_H 0,86 (3H, t, *J* = 7.0 Hz, Me-29) ppm. One olefinic and oxygenated methine (CH-OH) signals were also observed in ^{1}H-NMR at δ_H 5.34 (1H; br. d; *J* = 4.8 Hz; H-6) and δ_H 3.50 (1H; sept; J = 10.2 Hz; 3.7 Hz, H-3) ppm, respectively. The ^{13}C NMR ($CDCl_3$, 150 MHz) spectrum showed 30 carbon resonances, which were classified by their chemical shifts, DEPT and HMQC spectra as 6 methyls (2 tertiary at δ_C 11.9 and 19.5; 3 secondary at δ_C 18.9, 19.4, 19.9; 1 primary at 12.1) ppm, 12 methylenes at δ_C 37.3, 31.7, 42.3, 31.7, 21.2, 39.8, 24.4, 28.3, 34.0, 29.8, 26.0 and 23.1 ppm, 9 methines at δ_C 31.9, 50,2, 56.8, 56.1, 36.2, 45.9, 29.2 (1 oxygenated at

δ_C 71.9 and 1 olefinic carbons at 121.8) ppm and 3 quaternary carbons at δ_C 36.6, 42.3 (1 olefinic at δ_C 140.8) ppm. These functionalities accounted for 1 out of the total 5 hydrogen deficiency index. The remaining four hydrogen deficiency index were consistent with the tetracyclic structure. These are characterictic resonances of sterol, with one double bond like *β*- sitosterol or stigmas-5-en-3*β*-ol previously published, but found the addition of 1 methylene which resonates at 29.8 in compound **2** that can be seen clearly in the C and DEPT spectra, which was not found in sitosterol (Martínez et al., 2017). The selected ^{1}H-^{1}H COSY spectrum of **2** (**Figure 2**), 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 functional group position of **2** was deduced from the HMBC spectra (**Figure 2**). In the HMBC spectrum, there were correlations between H-19 (CH_3-19 δ_H 0.99) to a quaternary C sp^2 C-5 (δ_C 140.8); C-1 (δ_C 37.3); C-9 (δ_C 50.2) and C-10 (δ_C 36.6) these correlations indicating that CH_3-19 embeded at C-10. Some HMBC cross peaks were observed from H-18 (CH_3-18 δ_H 0.67) to C-12 (δ_C 39.8); C-17 (δ_C 56.1)and C-13 (δ_C 42.3) these correlations suggesting that CH_3-18 embeded at C-13. The methylene protons at δ_H 1.25 and 1.21 (H-28) correlate to the methine at δ_C 45.9 (C-24) and with carbon methyl δ_C 12.1 (C-29).

Table 1. NMR data for steroids **1** and reference compound

No.	Steroid **1** CD_3Cl, 600MHz		β-sitosterol acetate,(El-kader *et al*., 2012) (CD_3OD, 600MHz)	
	δ_C (mult.) ppm	δ_H (Integ, mult, *J*= Hz) ppm	δ_C (mult.) ppm	δ_H (Integ, mult, *J*= Hz) ppm
1	37.4 (t)	1.13 (1H, m); 1.87 (1H, m)	37.29	
2	28.2 (t)	1.58 (1H, m); 1.87 (1H, m)	30.62	
3	74.2 (d)	4.60 (1H, m)	77.79 (d)	3.45 (1H, m)
4	38.3 (t)	2.13 (1H, m); 2.31 (1H, m)	39.67	2.13 (1H, m); 2.32 (1H, br.s)
5	139.9 (s)	-	142.86 (s)	-
6	122.9 (d)	5.37 (1H; d; 6)	122.76 (d)	5.37 (1H; br.s; 4.8)
7	32.1 (t)	1.54 (1H, m); 1.97 (1H, m)	32.92 (t)	
8	32.1 (d)	1.45 (1H, m)	32.92 (d)	
9	50.3 (d)	0.94 (1H, m)	49.42 (d)	
10	36.8 (s)	-	37.29 (s)	-
11	21.2 (t)	1. 46 (1H, m); 1.61 (1H, m)	21.63 (t)	1.24-2.03
12	39.9 (t)	1.17 (1H,m); 2.00 (1H,m)	39.67 (t)	(m, other CH and CH2)
13	42.5 (s)	-	42.13 (s)	-
14	56.8 (d)	1.00 (1H, m)	58.34 (d)	
15	24.5 (t)	1.08 (1H, m); 1.58 (1H, m)	25.92 (t)	1.24-2.03
16	28.4 (t)	1.20 (1H, m); 1.84 (1H, m)	30.26 (t)	(m, other CH and CH2)
17	56.2 (d)	1.11 (1H, m)	57.56 (d)	
18	12.0 (q)	0.68 (3H, s)	12.23 (s)	0.72 (3H, d, J=7,5)
19	19.5 (q)	1.01 (3H, s)	19.32 (s)	1.05 (3 H, s)
20	36.3 (d)	1.35 (1H, m)	37.29 (d)	
21	19.0 (q)	0.81 (3H; d; 6)	19.32 (q)	1.00 (3H, d; 6.2)
22	34.1 (t)	1.31 (1H, m); 1.00 (1H, m)	34.98 (t)	
23	26.2 (t)	1.17 (1H, m); 1.61 (1H, m)	25.92 (t)	
24	46.0 (d)	0.92 (1H, m)	47.43 (d)	
25	29.3 (d)	1.67 (1H, m)	30.26 (d)	
26	19.2 (q)	0.81 (3H; d; 6.2)	20.07 (q)	0.85 (3H, d; 6.8)
27	20.0 (q)	1.00 (3H; d; 6.2)	21.63 (q)	0.88 (3H, d, J=7.5)
28	23.3 (t)	1.23 (1H, m); 1.32 (1H, m)	24.02 (t)	
29	12.2 (q)	0.78 (3H; t; 1.9)	14.36 (t)	0.90 (3H, d, J=7.5)
COO-**CH**$_3$	21.6 (q)	2.04 (3H, s)	20.07 (q)	2.05 (3H, s)
COO-CH_3	170.8	–	175.20	

The methyl protons at at δ_H 0.90 (H-21) correlate to the methine at δ_C 56.1 (C-17) and with carbon δ_C 36.2 (C-20). The HMBC correlations from the tertiary, secondary and primary methyl protons to their neighbor carbons, enabled the assignment of the two tertiary methyls at C-10 and C-13, secondary methyl at C-20 and C-25 (2×) as well as a primary methyl at C-29, respectively.

Furthermore, the olefinic proton, δ_H 5.34 (1H, br d, J = 4.8 Hz, H-6) was correlated to methylene carbon at C-4 (δ_C 38.3) and C-7 (δ_C 32.1), quaternary carbon at C-10 (δ_C 36.8) indicating an olefinic moiety was located at C-5 and C-6 ($\Delta^{5,6}$). Correlations from methylene protons, H-4 δ_H 2.25(2H, m) and H-1 δ_H 1.84 (1H, m) with C-3 (δ_C 71.9), were used to assign an hydroxyl group which was located on C-3. The HMBC spectrum of **2** exhibited interaction of C-23a at δ_C 26.0 with H-28 at δ_H 1.25 and 1.21 and with H-23 at δ_H 1.23. Correlations also shown from methylene protons, H-23a δ_H 1.13 (2H, m) with methine carbon at δ_C 45.9 (C-24), than from methylene protons H-23 at δ_H 1.23 (2H, m) with methylene carbon at δ_C 26.0 (C-23a). These HMBC correlation with The ^{13}C NMR, DEPT and COSY spectrum defined the presence of sp^3 methylene carbon C-23 at δ_C 29.8 and C-23a at δ_C 26.0. The ^{1}H NMR and ^{13}C NMR spectral data were compared with β-sitosterol, these data suggested the presence of Δ^5 steroid-type like stigmas-5-en-3β-ol with had one double bond previously published (Cayme & Ragasa, 2004). The similar signals except

Table 2. NMR data for Steroids **2** and reference compound

No	Steroid **2** (23a-Homostigmast-5en-3β-ol)		, 23a-Homostigmast-5en-3β-ol (400MHz, $CDCl_3$). (Naz *et al*., 2013)	
	δ_C (ppm) (mult.)	δ_H (ppm) (∑H, m, J)	δ_C (ppm) (mult.)	δ_H (ppm) (∑H, m, J)
1	37.3 (t)	1.05 (1H, m);1.84(1H,m)	37.3	1.06 (1H, m);1.81(1H, m)
2	31.7 (t)	1.54 (2H, m)	31.9	1.52 (2H, m)
3	71.9(d)	3.50 (1H, sept, 3,7;10,2)	71.8	3.50 (1H, sept)
4	42.3 (t)	2.25 (2H, m)	42.3	2.25 (2H, m)
5	140.8(s)	-	140.8	-
6	121.8 (d)	5.34 (1H, br d, J = 4,8 Hz)	122.1	5.34 (1H, brd, J=4,8Hz)
7	31.7 (t)	1.93 (1H, m); 1.84 (1H, m)	31.7	1.93 (1H, m); 1.84 (1H,m)
8	31.9 (d)	1.97 (1H, m)	31.9	1.98(1H, m)
9	50.2 (d)	0.91 (1H, m, H-9),	50.2	0.91(1H, m)
10	36.6 (s)	-	36.5	-
11	21.2 (t)	1.46 (2H, m)	21.1	1.45(2H, m)
12	39.8 (t)	1.13 (2H, m)	39.8	1.14 (2H, m)
13	42.3 (s)	-	42.3	-
14	56.8 (d)	1.06 (1H, m)	56.9	1.06 (1H, m)
15	24.4 (t)	1.50 (1H, m)	24.4	1.54 (1H, m)
16	28.3 (t)	1.19 (2H, m)	28.2	1.16 (2H, m)
17	56.1 (d)	1.10 (1H, m)	55.0	1.10 (1H, m)
18	11.9 (s)	0.67 (3H, s)	12.0	0.75 (3H, s)
19	19.5 (s)	0.99 (3H, s)	19.4	0.80(3H, s)
20	36.2 (d)	1.45 (1H, m)	36.2	1.43 (1H, m)
21	18.9 (q)	0.90 (3H, d, 8,0 Hz)	18.8	0.99(3H, d, J= 8,0 Hz)
22	34.0 (t)	1.50(1H, m); 1.22 (1H, m)	34.0	1.51(1H,m); 0.99(1H,m)
23	29.8 (t)	1.23 (2H, m)	29.7	1.52(2H, m)
23a	26.0(t)	1.13 (2H, m)	26.1	1.12 (2H, m)
24	45.9 (d)	0.93 (1H, m)	45.9	0.91(1H,m)
25	29.2 (d)	1.80 (1H, m)	29.3	1.81(1H,m)
26	19.4 (q)	0.82 (3H, d, 6,8 Hz)	19.0	0.79 (3H, d, J=6,8)
27	19.9 (q)	0.83 (3H, d, 6,8 Hz)	19.8	0.83 (3H, d, J= 6.8)
28	23.1 (t)	1.25 (1H, m); 1.20 (1H, m)	23.1	1.23(2H, m)
29	12.1 (q)	0.84 (3H, t, 7,0 Hz)	12.1	0.86(3H, t, J=7,0 Hz)

for marked difference in the addition of one methylene carbon at C-23, so named C-23a-Homo after C-23 at [δ_C 26.0, δ_H 1.13, (2H, m, H-23a)], so compound **2** steroid-type cholestan named 23a-Homostigmast-5-en-3β-ol. A detailed comparison of the ^{1}H NMR and ^{13}C NMR spectral data of compound **2** were similar to those of the 23a-Homostigmast-5-en-3*β*-ol which were previously isolated from n-hexane fraction of the roots of *Fumaria parviflora* (Naz *et al*., 2013).

The structures of steroid compounds that have been isolated from the genus Aglaia compared to steroids **1** and **2** have differences in the basic structure and in their side groups. Steroid compounds that have been found from the genus Aglaia include, pregnan-type steroids, pregnasetal from *A. silvestris* (Pointinger et al., 2008). (Weber et al., 2000) have succeeded in isolating androstan-type steroid compounds, as well as cholestan-type steroid compounds, from the leaves of *A. rubiginosa*. (Harneti et al., 2014), had succeeded in finding steroid compound from the stem bark of *A. eximia* and showed cytotoxic activity against murine leukemia P-388 cells with an IC_{50} value of 11.42 *μ*g/mL. (Farabi et al., 2017) have succeeded in finding a steroid (β-sitosterol) from the stem bark of *A. argentea*. (Farabi et al., 2018), have also succeeded in finding pregnane-type steroids from the stem bark of *A. elliptica*. Steroids **1** and **2** showed the weak activity against MCF-7.

CONCLUSIONS

Two cholestan type steroid compounds were investigated from ethyl acetate and *n*-hexane extract of *Aglaia angustifolia* Miq stem bark and identified as a Stigmast-5en-3*α*-acetat (**1**), as a new steroid with *α*-sterochemistry of acetyl moety at C-3 and 23a-Homostigmast-5en-3*β*-ol (**2**), a steroid with unique side chain moiety. The isolated compounds showed weak activity against MCF-7 breast cancer cell lines.

ACKNOWLEDGMENTS

This investigation was financially supported by Indonesia Endowment Fund for Education (LPDP). Doctoral Grant number, PRJ 5513/LPDP.3'2016, by R. P. H. Facility support was also provided by Research Center for Pharmaceutical Ingredients and Traditional Medicine, National Research and Innovation Agency (BRIN), and by LP_2M Universitas Nusa Bangsa, Bogor.

REFERENCES

Agarwal, G., Chang, L. S., Soejarto, D. D., & Kinghorn, A. D. (2021). Update on phytochemical and biological studies on rocaglate derivatives from Aglaia species. *Planta Medica*, *87*(12–13), 937–948. https://doi.org/10.1055/a-1401-9562

Awang, K., Loong, X., Hoong, K., Supratman, U., Litaudon, M., Mukhtar, M. R., & Muhamad, K. (2012). Triterpenes and steroids from the leaves of *Aglaia exima* (Meliaceae), *Fitoterapia*, *83*(8), 1391–1395. https://doi.org/10.1016/j.fitote.2012.10.004

Cayme, J.-M. C., & Ragasa, C. Y. (2004). Structure elucidation of β-stigmasterol and β-sitosterol from *Sesbania grandiflora* [Linn.] Pers. and β-carotene from Heliotropium indicum Linn. by NMR spectroscopy. *Kimika*, *20*, 5–12.

El-kader, A. M. A., Ahmad, A. S., Nafady, A. M., & Ibraheim, Z. Z. (2012). Xanthones and lignan glycosides from the aerial parts of *Polygonum bellardii* all growing in Egypt. *Pharmacognosy Magazine.*, *9*(34), 135–143.

Farabi, K., Harneti, D., Nurlelasari, Maharani, R., Hidayat, A. T., Awang, K., Supratman, U., & Shiono, Y. (2018). New cytotoxic pregnane-type steroid from the stem bark of *Aglaia elliptica*. *Records of Natural Product*, *2*, 121–127.

Farabi, K., Harneti, D., Nurlelasari, Maharani, R., Hidayat, A. T., Supratman, U., Awang, K., & Shiono, Y. (2017). Cytotoxic steroids from the bark of *Aglaia argentea* (Meliaceae) cytotoxic steroids from the bark of *Aglaia argentea* (Meliaceae). *CMU Journal of Natural Sciences*, *16*(4), 293–306. https://doi.org/10.12982/CMUJNS.2017.0024

Gu, B. Bin, Wu, W., Jiao, F. R., Jiao, W. H., Li, L., Sun, F., Wang, S. P., Yang, F., & Lin, H. W. (2018). Aspersecosteroids A and B, two 11(9 → 10)-abeo-5,10-secosteroids with a dioxatetraheterocyclic ring system from *Aspergillus flocculosus* 16D-1 [Rapid-communication]. *Organic Letters*, *20*(24), 7957–7960. https://doi.org/10.1021/acs.orglett.8b03530

Harneti, D., & Supratman, U. (2021). Phytochemistry and biological activities of *Aglaia species*. *Phytochemistry*, *181*(April 2020). https://doi.org/10.1016/j.phytochem.2020.112540

Harneti, D., Supriadin, A., Ulfah, M., Safari, A., Supratman, U., Awang, K., & Hayashi, H. (2014). Cytotoxic constituents from the bark of *Aglaia eximia* (Meliaceae). *Phytochemistry Letters*, *8*, 28–31. https://doi.org/10.1016/j.phytol.2014.01.005

Hutagaol, Ricson P, Rahadian, I., Harneti, D., Hidayat, A. T., & Awang, K. (2020). A triterpenoid from *Aglaia angustifolia* Miq stem bark. *Research Journal of Chemistry and Environment*, *24*(3), 41–44.

Hutagaol, R.P., Harneti, D., Safari, A., Hidayat, A. T., Supratman, U., Awang, K., & Shiono, Y. (2021). Cytotoxic triterpenoids from the stem bark of *Aglaia angustifolia*. *Journal of Asian Natural Products Research*, *1*(8), 1–8. https://doi.org/10.1080/10286020.2020.1776704

Hutagaol, R.P., Supratman, U., Rahadian, I., -, S., Harneti, D., Hidayat, A. T., Awang, K., & Shiono, Y. (2022). A nortriterpenoid and steroid

from the stem bark of *Aglaia angustifolia* Miq (Meliaceae). *Jurnal Kimia Valensi*, *8*(1), 106–112. https://doi.org/10.15408/jkv.v8i1.23011

Katja, D. G., Farabi, K., Nurlelasari, N., Harneti, D., Julaeha, E., Hidayat, A. T., Mayanti, T., Maharani, R., Supratman, U., & Awang, K. (2017). Cytotoxic steroids from the stem bark of *Chisocheton cumingianus* (Meliaceae). *Molekul*, *12*(1), 1-7. https://doi.org/10.20884/1.jm.2017.12.1.257

Kikuchi, T., Horii, Y., Maekawa, Y., Masumoto, Y., In, Y., Tomoo, K., Sato, H., Yamano, A., Yamada, T., & Tanaka, R. (2017). Pleurocins A and B: Unusual 11(9 → 7)-abeo-ergostanes and eringiacetal b: A 13,14-seco-13,14-epoxyergostane from fruiting bodies of *Pleurotus eryngii* and Their inhibitory effects on nitric oxide production. *Journal of Organic Chemistry*, *82*(19), 10611–10616. https://doi.org/10.1021/acs.joc.7b01259

Kim, W. S., Du, K., Eastman, A., Hughes, R. P., & Micalizio, G. C. (2018). Synthetic nat-or ent-steroids in as few as five chemical steps from epichlorohydrin. *Nature Chemistry*, *10*(1), 70–77. https://doi.org/10.1038/NCHEM.2865

Lailatul, Q., & Nurrachma, M. Y. (2020). Pemilihan sel yang tepat untuk penelitian kanker payudara (Selection of appropriate cells for breast cancer research). *BioTrends*, *11*(2), 17–28.

Li, J. F., Ji, K. L., Sun, P., Cai, Q., Zheng, X. L., Xiao, Y. D., Cao, D. H., Xiao, C. F., Zhang, Z. Y., Li, X. N., Hu, H. Bin, Yu, Z. Y., & Xu, Y. K. (2021). Structurally diverse steroids with nitric oxide inhibitory activities from *Aglaia lawi*i leaves. *Phytochemistry*, *183*, 112651. https://doi.org/10.1016/j.phytochem.2020.112651

Martínez, M., Benítez, B., Alvarez, S., Prieto, R., Valdo, G., Da, J., Bravo, J. A., & Vila, J. L. (2017). Determination of an active principle of *Polygonum punctatum* elliot ; full NMR spectra of stigmast-5-En-3 □ -Ol. *Bolivian Journal of Chemistry*, *34*(1), 14–27.

Naz, I., Saifullah, & Khan, M. R. (2013). Nematicidal activity of nonacosane-10-ol and 23a-homostigmast-5-en-3β- ol isolated from the roots of *Fumaria parviflora* (Fumariaceae). *Journal of Agricultural and Food Chemistry*, *61*(24), 5689–5695. https://doi.org/10.1021/jf401309r

Pointinger, S., Promdang, S., Vajrodaya, S., Pannell, C. M., Hofer, O., Mereiter, K., & Greger, H. (2008). Silvaglins and related 2,3-secodammarane derivatives – unusual types of triterpenes from *Aglaia silvestris*. *Phytochemistry*, *69*(15), 2696–2703. https://doi.org/10.1016/j.phytochem.2008.08.025

Ratnaweera, P. B., Williams, D. E., Patrick, B. O., De Silva, E. D., & Andersen, R. J. (2015). Solanioic acid, an antibacterial degraded steroid produced in culture by the fungus *Rhizoctonia solani* isolated from tubers of the medicinal plant *Cyperus rotundus*. *Organic Letters*, *17*(9), 2074–2077. https://doi.org/10.1021/acs.orglett.5b00596

Ren, Y. H., Liu, Q. F., Chen, L., He, S. J., Zuo, J. P., Fan, Y. Y., & Yue, J. M. (2019). Urceoloids A and B, two C19 steroids with a rearranged spirocyclic carbon skeleton from *Urceola quintaretii*. *Organic Letters*, *21*(6), 1904–1907. https://doi.org/10.1021/acs.orglett.9b00539

Supriatno, Herlina, T., Harneti, D., Maharani, R., Tatang, A., Mayanti, T., Supratman, U., & Azmi, M. N. (2018). A new limonoid from stem bark of *Chisocheton pentandrus* (Meliaceae). *Natural Product Research*, *32*, 2610–2616. https://doi.org/10.1080/14786419.2018.1428600

Weber, S., Puripattanavong, J., Brecht, V., & Frahm, A. W. (2000). Phytochemical investigation of *Aglaia rubiginosa*. *Journal of Natural Product*, *63*, 636–642.

Zhang, H., Xu, H., Song, Z., Chen, L., & Wen, H. (2012). Molluscicidal activity of *Aglaia duperreana* and the constituents of its twigs and leaves. *Fitoterapia.*, *83*, 1081–1086.