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A Nortriterpenoid and Steroid from the Stem Bark of *Aglaia angustifolia* Miq (Meliaceae)

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Abstract

A nortriterpenoid, 3-*epi*-cabraleahydroxylactone (**1**) and a steroid, stigma-4-en-3-on **(2)** were isolated from the *n*-hexane extract of stem bark of *Aglaia angustifolia* Miq. Compound (**2**) was isolated for the first time from this Genus. The structure of both compounds were identified by spectroscopic datas including one and two-dimensional NMR as well as infrared spectrum, high-resolution mass spectrometric analysis and by comparing with those spectral data previously.

Keywords: *Aglaia angustifolia*, Cabraleahydroxylactone, Stigmastane, Nortriterpenoid.

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1. INTRODUCTION

Aglaia, the largest genus of subtropical and tropical angiosperm family of Meliaceae, consists of 130 species distributed mainly in the Southern mainland China, Indo-Malaysian region and the Pacific Island (Mabberley & Pannel, 1995), (Inada *et al*., 2001). Meliaceae is a family known for the presence of triterpenes with interesting biological activities, such as hypoglycemia, anticancer, anti-inflammatory, antifeedant, insecticides, and antitumor activities. The plants genus *Aglaia* has been found to yield a variety of different classes of compounds such as triterpenoid, lignans, rocaglate derivatives, sesquiterpenoids, tetraterpenoids and steroids (Liu *et al*., 2014); (Weber *et al*., 2000); (Pan *et al*., 2010).

Aglaia angustifolia (Miq.) species mainly distributed in Indonesia (Sumatera, Kalimantan islands) and declared almost extinct (Pannel, 1992). We found that *A. angsutifolia* (Miq.) has not done much phytochemical research. The course of our continuous investigation for biologically active compounds from Indonesian *Aglaia* plants, we already isolated several active compounds from *A. Smithii* (Harneti *et al*., 2012); *A. eximia* (Harneti *et al*., 2014); (Sianturi, *et al*., 2015a); (Sianturi, *et al*., 2015b); *A. argentea* (Farabi, *et al*., 2017a); (Farabi, *et al*., 2017b); *A. elliptica* (Farabi *et al*., 2018), (Hidayat *et al*., 2017) and recently *A. angustifolia* (Hutagaol *et al*., 2021); (Hutagaol *et al*., 2020a) (Hutagaol, *et al*., 2020b).

In this paper, we report the isolation and structural elucidation of a nortriterpenoid, 3-*epi*-cabraleahydroxylacton, and a steroid stigmast-4-en-3-on which to our knowledge is a new type-steroid compound found in *Aglaia* genus.

2. MATERIAL AND METHODS

General

IR spectra were measured on a Perkin Elmer 1760 X FT-IR in potassium bromide

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pellet (Waltman, MA, USA). 1D-NMR and 2D-NMR spectra were recorded with a Bruker 600 MHz/Topspin 3.5P17 spectrometer for **2** and JEOL JNM ECZ-600 spectrometer for **1,** both using (Tetra Methyl Silane)(TMS) as an internal standard. Mass spectra were obtained with a Water Qtof, HR-MS XEV^{otm} mass spectrometer (Waters, Milford, MA, USA). Technical solvents were distilled prior to use for maceration, isolation and spectral grade solvents were employed for spectroscopic measurements. Column chromatography (CC) was carried out on silica gel 60 (70–230 mesh and 230–400 mesh) Merck (Merck, Darmstadt, Germany), and Octa Desyl Silane (ODS, Fuji Silsya). TLC on silica gel Merck 60 GF_{254}, 0,25mm (Merck, Darmstadt, Germany). Spots were visualized under UV light at is lambda 254 nm and 365 nm, simultaneously by spraying with sulfuric acid 10% in ethanol and vanillin reagent followed by heating

Plant Material

The stem bark of *A. angustifolia* (Miq.) was collected from Bogor Botanical Garden, Indonesian Science Institute, West Java Indonesia in February, 2017. A voucher specimen (II.K.57a) was deposited at the herbarium.

Plant Extraction

Dried powder stem bark (1.97 kg) of *A. angustifolia* was extracted with *n*-hexane exhaustively at room temperature (3 x 24 hours, each 3 L). The solution was decanted and then evaporated to give a residue of 25 g of *n*-hexane extract. The *n*-hexane (24 g) extract was subjected to vacuum liquid chromatography (VLC) over a silica gel using gradient elution of *n*-hexane-ethyl acetate (EtOAc)-methanol (MeOH) as a eluent to afford 7–fractions (A-G). Fraction C (5.02 g) was further subjected to column chromatography (CC) using silica gel (70-230 mesh) and using 1% gradient mixture of *n*-hexane-EtOAc (10:0 - 7:3) and followed by 10% gradient (7:3 - 0:10)as eluting solvents to afford, 10 subfractions (C1-C10) fraction C8 (322.4 mg) was subjected to CC on silica gel G (70-230 mesh), eluted with isocratic mixture *n*-hexane : EtOAc = 8:2 to give 8 subfractions (C8A – C8H) were obtained. Subfractions C8C, C8D, C8E were further purified by recrystallization using MeOH gave white solid compound **1** (17.1 mg).

Furthermore fraction B (2.58 g) results was subjected to CC over silica gel (70-230 mesh) using 2% gradient mixture of *n*-hexane-EtOAc-MeOH as eluting solvent to afford 20 sub fractions (B1-B20). Sub fraction B5 (390.7 mg) was subjected to CC on Silica gel (70-230 mesh) using isocratic mixture 95:5 = *n*-hexane –EtOAc eluent to give three combined subfractions (B5A-B5C). Subfraction B5C (149.7 mg) was subjected to CC over ODS using a isocratic mixture MeOH - H_2O =95:5 as a eluent to compound **2** (9.1 mg).

Spectroscopic Data of Compound 1

White amorphous solid, IR (KBr) ν_{max} cm^{-1}: 3469 strong (O-H stretch), 2947 strong (C-H sp^3 stretch), 1750 (C=O carbonyl), 1387 medium (C-H aliphatic bent), 1250 medium (*gem*-dimethyl), 1073 strong (C-O stretch); HRTOF-MS: *m/z* 417.3414 $[M+H]^+$, calculated *m/z* $[C_{27}H_{45}O_3]^+$ 417.3369. ^{1}H-and ^{13}C-NMR data: see **Table 1**, which is compared with another related data of dammarane tris-nortriterpenoid.

Spectroscopic Data of Compound 2

White amorphous solid, IR (KBr) ν_{max} cm^{-1}: 342 weak (C-H sp^2 stretch), 2936 and 2868 strong (C-H sp^3 stretch), 1680 (C=O conjugated carbonyl), 1464 and 1381 medium (C-H aliphatic bent); HRTOF MS: *m/z* 413.3787$[M+H]^+$, calculated *m/z* $[C_{29}H_{49}O]^+$ 413.3783, ^{1}H-and ^{13}C-NMR data: see **Table 1**, which is compared with another related data of steroid compound.

Table 1. ^{1}H and ^{13}C NMR of compound **1** and **2** and related data.

No.	Compound 1		Cabraleahydroxylacton		Compound 2		Stigmast-4-en-3on	
	$CDCl_3$, ^{1}HNMR 600 MHz, ^{13}C NMR 150 MHz		$CDCl_3$, ^{1}H NMR 400MHz, ^{13}C NMR 100MHz.(Phongmaykin *et al*., 2008)		$CDCl_3$,^{1}H NMR 600 MHz, ^{13}C NMR 150MHz		$CDCl_3$,^{1}H NMR 400MHz, ^{13}C NMR 100MHz (Kolak *et al*., 2005)	
	δ_H (integral, mult, *J* Hz)	δ_C	δ_H (integral, mult, *J* Hz)	δ_C	δ_H (integral, mult, *J* Hz)	δ_C	δ_H (integral, mult, *J* Hz)	δ_C
1.	1.27 (1H, m) 1.35 (1H, m)	34.0	1.29 (1H, m) 1.38 (1H, m)	33.6	1.62 (1H, m) 1.96 (1H, m)	35.6	1.60 (1H, m) 1.94 (1H,m)	35.6
2.	1.53 (1H, m) 1,90 (1H, m)	25,8	1.55 (1H, m) 1.92 (1H, m)	25.4	2.29 (1H, m) 2.35 (1H, m)	34.0	2.25 (1H, m) 2.35 (1H, m)	34.0
3.	3.37 (1H, s)	76.3	3.38(1H, t, 2.7)	76.2	-	198.7	-	199.7
4.	-	37.2	-	37.6	5.65 (1H, s)	122.7	5.74 (1H, s)	123.7
5.	1.25 (1H, m)	49.3	1.27 (1H, m)	49.3	-	170.7	-	171.0
6.	1.37 (2H, m)	18.1	1.41(2H, m)	18.2	2.21 (1H, m) 2.32 (1H, m)	32.9	2.23 (1H, m) 2.30 (1H, m)	32.9
7.	1.71 (2H, m)	26.9	1.24 (1H, m) 1.57 (1H, m)	25.1	0.96 (1H, m) 1.77 (1H, m)	32.1	0.96 (1H, m) 1.74 (1H, m)	32.1
8.	-	40.5	-	40.3	1.44 (1H, m)	35.7	1.42 (1H, m)	35.7
9.	1.41 (1H, dd; 2.4, 13.2)	50.4	1.44 (1H, m)	50.3	0.85 (1H, m)	53.8	0.86 (1H, m)	53.8
10.	-	37.6	-	37.2	-	38.6	-	38.6
11.	1.20 (1H, m) 1.70 (1H, m)	25.3	1.20 (1H, m) 1.73 (1H, m)	26.8	1.22 (1H, m) 1.78 (1H, m)	28.2	1.22 (1H, m) 1.79 (1H, m)	28.0
12.	1.26 (1H, m) 1.71 (1H, m)	21.2	1.21 (1H, m) 1.73 (1H, m)	21.3	1.10 (1H, m) 1.97 (1H, m)	39.6	1.12 (1H, m) 1.79 (1H, m)	39.6
13.	1.53 (1H, m)	43.1	1.60 (1H, m)	43.1	-	42.4	-	42.4
14.	-	50.3	-	50.5	0.94 (1H, m)	55.9	0.94 (1H, m)	55.8
15.	1.11 (1H, ddd 12, 8, 1.5) 1.49 (1H, m)	31.1	1.10 (1H, ddd, 11.9, 8.6, 1.5) 1.48 (1H, m)	31.1	1.38 (1H, m) 1.54 (1H, m)	24.2	1.36 (1H, m) 1.54 (1H, m)	24.1
16.	1.25 (1H, m) 1.78 (1H, m)	25.0	1.29 (1H, m) 1.80 (1H, m)	25.0	1.22 (1H, m) 1.78 (1H, m)	28.2	1.22 (1H, m) 1.78 (1H, m)	28.1
17.	1.23 (1H, m)	49.4	1.97 (1H, td, 10.7, 6.1)	49.5	1.05 (1H, m)	56.0	1,07 (1H,m)	56.1
18.	0.92 (3H, s)	15.4	0.94 (3H, m)	15.5	0.64 (3H, s)	11.9	0.72 (3H, s)	11.9
19.	0.82 (3H, s)	16.0	0.83 (3H, m)	16.0	1.11 (3H, s)	17.4	1.19 (3H, s)	17.4
20.	-	90.3	-	90.2	1.28 (1H, m)	36.1		36.1
21.	1.33 (3H, s)	25.4	1.34 (3H, s)	25.4	0.74 (3H, d, 6.7)	18.7	0.90 (3H, d, 6.6)	18.7
22.	1.94 (1H, m) 2.01 (1H, m)	31.1	1.93 (1H, m) 2.10 (1H, ddd, 12.8 10.1, 9.2)	31.2	0.94 (1H, m) 1.25 (1H, m)	33.9	0.94 (1H, m) 1.25 (1H, m)	33.8
23.	2.52 (1H, d, 10) 2.62 (1H,d; 9.9)	29.3	2.53(1H, ddd, 18.0 10.2, 4.6) 2.62 (1H, ddd, 18.0, 10.2, 9.0)	29.2	1.08 (1H, m) 1.09 (1H, m)	26.1	1.08 (1H, m) 1.09 (1H, m)	26.0
24.	-	176.9	-	176.8	0.85 (1H, m)	45.9	0.85 (1H, m)	45.8
25.	-		-	-	1.60 (1H, t)	29.2	1.60 (1H, m)	29.1
26.	-		-	-	0.84 (3H, d, 6.5)	19.8	0.83 (3H, d, 6.8)	19.2
27.	-		-	-	0.79 (3H, d, 6.5)	19.0	0,80 (3H, d, 6.8)	19.8
28.	0.91 (3H, s)	28.4	0.92 (3H,s)	28.3	1.18 (2H, m)	23.1	1.16 (2H, m)	23.1
29.	0.81 (3H, s)	22.1	0.82 (3H,s)	22.1	0.77 (1H, m)	11.9	0.85 (3H, t)	11.9
30.	0.87 (3H, s)	16.3	0.88 (3H,s)	16.3				

3. RESULTS AND DISCUSSION

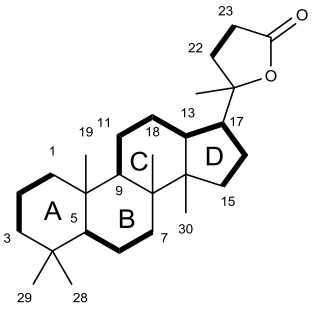
Compound **1**, was obtained as a white amorphous solid, HRTOF MS spectral data showed an $[M+H]^+$ ion peak at *m/z* 417.3414 (calcd. $[C_{27}H_{45}O_3]^+$ 417.3369) which corresponded to the molecular formula of $C_{27}H_{44}O_3$. The IR spectrum showed absorption peaks at 3476; 2943; 1716; 1470; 1250 and 1073 cm^{-1} suggesting the presence of hydroxyl, aliphatic carbon-hydrogen, carbonyl,

gem-dimethyl, and ether group respectively. The ^{1}H-NMR spectrum showed six peaks corresponding to six tertiary methyls at δ_H 0.92 (Me-18), 0.82 (Me-19), 1.33 (Me-21), 0.91 (Me-28), 0.81 (Me-29) and 0.87 (Me-30). Other spectra showed one signal oxygenated methane at δ_H 3.37 (H-3). These signals strengthen existence of nor-triterpenoid skeleton (Phongmaykin *et al.*, 2008).

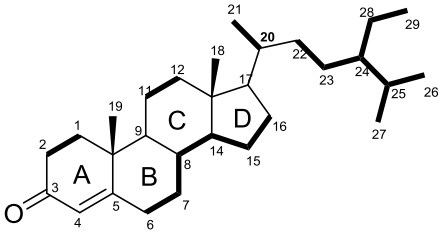
The ^{13}C NMR and DEPT 135^{o} (150MHz, $CDCl_3$) spectra showed peaks corresponding to twenty seven carbons, consisting of six methyls, ten methylenes and five methines. The presence of six methyls at δ_C [15.6 (C-18), 16.1 (C-19), 25.4 (C-21), 28.4 (C-28), 22.2 (C-29), 16.4 (C-30)], one oxygenated methine at δ_C 76.3 (C-3), one oxygenated quaternary carbon at δ_C 90.3 (C-20) and one ester signal at 176.9 (C-24). These signals indicated release of the C-25, C-26 and C-27 atoms and the formation of the lactone ester at C24/20. The ^{13}C and DEPT signals showed possibility found five double bonds equivalence from pentacyclic dammarane of trisnor-triterpenoid skeleton. The ^{1}H-^{1}H COSY spectrum confirmed the dammarane triterpenoid skeleton consist of ring A, B, C, and D, showed by correlations of spectrum H-1/H-2/H-3, H-5/H-6/H-7, H-9/H-11/H-12/H-13, H-15/H-16/H-17 and H-22/H-23, see **Fig. 1**(**1**).

The HMBC spectrum confirmed the position of *gem*-dimethyl at C-4 by showing correlations of H-28 with the C-3, C-4, C-5, C-29 and correlations of H-29 with C-3, C-4, C-28. Those correlations also showed a ring on the dammarane. The position of lactone ring side chain at C-17 and methyl-21 at C-20 showed by correlations of H-21 with C-17, C-20, C-22. Correlations of H-22 and H-23 with C-20, and C-24 showed a lactone ring formation at C-23 and C-24 and loss of three C atoms at (C-25, C-26 and C-27), which also supported by ^{1}H-^{1}H COSY correlations spectrum. The HMBC spectrum showed correlations of H-3 with C-1, C-2, C-4, C-5 thus confirming hydroxy at C-3.

Then position of methyl-18 at C-8 and ring C of dammarane showed by correlations of H-18 with C-7, C-8, C-9 and C-14. Correlations of H-19 with C-1, C-2, C-5, C-9 and C-10 showed position of methyl-19 at C-10 and ring B of dammarane. Position of methyl-30 at C-14 ring D of dammarane showed by correlations of H-30 with C-8, C-13, C-14 and C-15. The DEPT, COSY, HSQC and HMBC spectra analysis allowed the complete assignment of all protons and carbons. Therefore, compound **1** was elucidated as 25,26,27-trisnor-damar-20(24)-olid-3-ol, see **Fig. 2(1).**

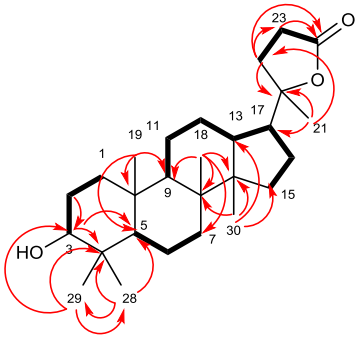


(**1**)



(**2**)

Figure 1. Ring of compound **1** and **2** based on ^{1}H-^{1}H COSY correlations



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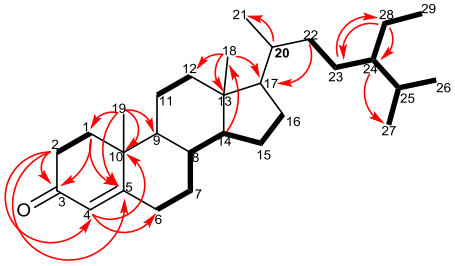
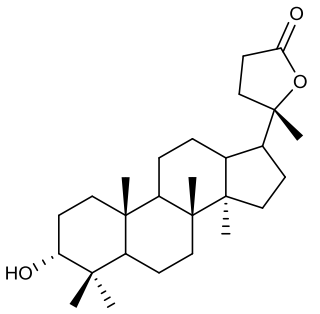


(**2**)

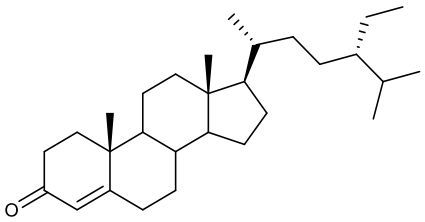
Figure 2. Selected HMBC and ^{1}H-^{1}H COSY correlations for compound **1** and **2**

Stereochemistry compound **1** obtained from the ^{1}H-NMR experiment showed that proton ^{3}J 0 Hz at C-3 indicated the –OH at C-3 as a α-oriented. Resonance value δ_C 90.3 at C-20 indicated stereochemistry 20*S* (Hawas *et al.*, 2013). Therefore, compound **1** was elucidated as 3-e*pi*-cabraleahydroxylactone, see **Fig. 3(1).**

Compound **2**, was obtained as a white amorphous solid, HRTOF MS spectrum showed an $[M + H]^+$ ion peak at *m/z* 413.3787 (calcd. 413.3783) which corresponded to the molecular formula of $C_{29}H_{48}O$. The IR spectrum showed absorption peaks at 3421; 2936; 2868; 1680, 1464 and 1381 cm^{-1} suggesting the presence of olefinic sp^2 carbon-hydrogen, aliphatic sp^3 carbon-hydrogen, carbonyl, aliphatic bent carbon-hydrogen respectively.



(**1**)



(**2**)

Figure 3. Structure of compound **1** and **2**

The ^{1}H-NMR (600MHz, $CDCl_3$) spectra showed six peaks corresponding to six tertiary methyls at δ_H 0.64 (Me-18), 1.11 (Me-19), 0.74 (Me-21), 0.84 (Me-26), 0.79 (Me-27) and 0.77 (Me-29). Other spectra showed one olefinic proton at δ_H 5.65 (H-4). These signals strengthen existence of steroid skeleton with olefinic at C-4. The ^{13}C NMR and DEPT 135^{o} (150MHz, $CDCl_3$) spectra showed peaks corresponding to twenty nine carbons, six methyls, eleven methylenes, eight methines and four quaternary carbons.

The presence of six methyls at δ_C [11.9 (C-18), 17.4 (C-19), 18.7 (C-21), 19.0 (C-26), 19.8 (C-27), 11.9 (C-29)] one sp^2 methine at δ_C 123.8 (C-4), two quaternary sp^3 carbons at δ_C [38.6 (C-10) and 42.2 (C-13), one quaternary carbonyl ketone signal at 199.7 (C-3). The ^{13}C and DEPT signals showed possibility found six double bonds equivalence, four from tetracyclic stigmastane-type of steroid skeleton and two others from one carbonyl and one olefinic (Chaturvedula & Prakash, 2012). The ^{1}H-^{1}H COSY spectrum confirmed the stigmastane-type of steroid skeleton consist of ring A, B, C, and D, showed by correlations of spectrum H-1/H-2, H-6/H-7/H-8/H-14/H-15/H-16, H-11/H-12, H-22/H-23/H-24/H-28/H-29, H-26/H-27, see **Fig. 1(2).**

The HMBC spectrum confirmed the position of carbonyl at C-3 showed by correlations of H-2 and H-1 with C-3. Correlations of H-2 with C-4 and H-1 with C-5 and H-4 with C-10 and C-6 confirmed the position of one double bound at C-4/C-5. Methyl-18 at C-13 showed by correlations of H-18 with C-17, C-12, and C-13. Correlations of H-19 with C-1 and C-10 showed position of methyl-19 at C-10. Position of side chain at C-17 ring D of steroid showed by correlations of H-22 and H-18 with C-17. Correlations of H-20 with C-21 confirmed position of methyl-21 at C-20. The HMBC spectrum showed correlations of H-23 with C-28, H-28 with C-23 and C-24, H-24 with C-27 confirmed the structure of steroid stigmastane side chain (Yayli & Baltaci, 1996), see **Fig. 2(2).**

Thorough analysis of the DEPT, COSY, HSQC and HMBC spectra allowed the complete assignment of all protons and carbons. Therefore, compound **2** was elucidated as a stigmast-4-en-3-on, which is compared with another related data of stigmastan-type steroid (Kolak *et al.*, 2005), see **Fig. 3(2)**.

4. CONCLUSION

Two secondary metabolite compounds were investigated from *n*-hexane extract of *Aglaia angustifolia* Miq stem bark and identified as a dammarane-type tris-nortriterpenoid,3-*epi*-cabraleahydroxylacton (**1**) and one stigmastan-type steroid, stigmast-4-en-3-on (**2**).

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