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STIGMASTANE-STEROID FROM THE BARK OF *Chisocheton lasiocarfus* (Meliaceae)

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ABSTRACT

Stigmastan-steroid, stigma-4-ene-3-on (**1**) has been isolated from the bark of *Chisocheton lansiocarpus*. The chemical structure of stigmastan-steroid was identified based on spectroscopic data and by comparison of spectral data obtained previously. The discovery of stigma-4-ene-3-on in *C. lansiocarpus* was shown in this study for the first time.

Keywords: *Chisocheton lansiocarpus*, Meliaceae., stigmastan-steroid, stigma-4-ene-3-on

INTRODUCTION

Phytosterols are a subgroup of the steroids, as an important class of bioorganic molecules, widespread in plants, animals, marines as well as fungi and have similarity to cholesterol in structure (Saeidnia et al., 2014), including β-sitosterol, campesterol, stigmasterol and cycloartenol (Ostlund, 2002). Phytosterols, especially β-sitosterol, reported have interesting activity including anti-inflammatory (Prieto et al., 2006), inducing apoptosis (Chai et al., 2008; Park et al., 2007; Ju et al., 2004), chemoprotective or chemopreventive effects (Ovesna et al., 2004), hypocholesterolemic (Zak et al., 1990), angiongenic effect (Moon et al., 1999), anti-diabetic (Gupta et al., 2011; Jamaluddin et al., 1994; Radika et al., 2013), and anti-oxidant (Baskar et al., 2012; Vivancos and Moreno, 2005).

Chisocheton genus, the second largest genus of the Meliaceae family, consists of more than 50 species and distributes in Nepal, India, Myanmar, South China, Thailand, Indonesia, Malaysia, and Papua New Guinea (Vossen and Umali, 2002). Previous phytochemical studies on *Chisocheton*plantsreported the presence of compounds with interesting biological activities including insecticidal limonoid (Roy and Sarat, 2004), antifungal meliacin-type compounds (Bordoloi et al., 1993), spermidine alkaloids (Tzouros et al., 2004), sesquiterpenoids (Phongmaykin et al., 2008),dammarane-type triterpenoids (Inada et al., 1993; Phongmaykin et al., 2008), tirucallane-type triterpenoids (Zhang et al., 2012; Yang et al., 2011), apo-tirucallane-type triterpenoids (Zhang et al., 2012), limonoids (Maneerat et al., 2008; Laphookhieo et al., 2008; Mohamad et al., 2009; Yang et al., 2009; Najmuldeen et al., 2010; Wong et al., 2011) and steroidsandphenolics(Phongmaykin et al., 2008).

As part of our studies on novelly occuring compounds from Indonesia *Chisocheton* plants, herein we isolated and determine the chemical structure stigmastane-steroid from the stem bark of *Chisocheton lasiocarfus* that have yet to be reported before.

MATERIAL AND METHODS

General Experimental Procedure

The IR spectra were recorded on a Perkin-Elmer 1760X FT-IR in KBr. Mass spectra were

obtained with a Water Qtof HR-MS XEV^{otm} mass spectrometer. ^{1}H- and ^{13}C-NMR spectra were obtained with a JEOL JNM A-500 spectrometer using TMS as an internal standard. Chromatographic separations were carried out on silica gel 60 andocta desyl silane (ODS, Fuji Silysia). TLC plates were precoated with silica gel GF_{254} (Merck, 0.25 mm), RP-18 (Merck, 0.25 mm), and detection was achieved by spraying with 10% H_2SO_4 in ethanol, followed by heating.

Plant material

The bark of *C. lasiocarfus*was collected in Bogor Botanical Garden, Bogor, Indonesia in June 2015. The plant was identified by the staff of the Bogoriense Herbarium, Bogor, Indonesia and a voucher specimen was deposited at the herbarium.

Plant extraction

Dried ground bark of *C. lasiocarfus*(4.1 kg) was extracted with methanol in room temperatur. Evaporation of the methanol extract in reduced pressure to produce the dark brown residue (209 g). The dark brown residue dissolved in water (1:1) and successively partitioned with *n*-hexane, EtOAc, and MeOH. Evaporation resulted in the crude extracts of *n*-hexane (10.5 g), EtOAc (20.0 g), and *n*-BuOH (50.0 g), respectively. The EtOAc extract (18.5 g) was subjected to vacuum liquid chromatography over silica gel using a gradient elution mixture of *n*-hexane-EtOAc (10:0-0:10) as eluting solvents to afford 10 fractions (A-H). Fraction C (2.5 g) was subjected to column chromatography over silica gel using a mixture of *n*-hexane:EtOAc (7:3) as eluting solvents to afford 6 fractions (C01-C06). Fraction C04 (85.5 mg) was subjected to column chromatography over silica gel using a mixture of *n*-hexane:Me_2CO (7:3) to give 5 fractions (D01-D05). Fraction D03 was separated by preparative TLC on silica gel GF_{254} using a mixture of *n*-hexane:Me_2CO (2:3) to give (Figure 1) (12.8 mg).

Stigma-4-ene-3-on (Figure 1). IR ν_{max} (cm^{-1}): 3050, 2960, 2830,1710 and 1606; ^{1}H NMR (500 MHz, $CDCl_3$), Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) Table 1; MS (*m/z* 413.3573 $[M+H]^+$).

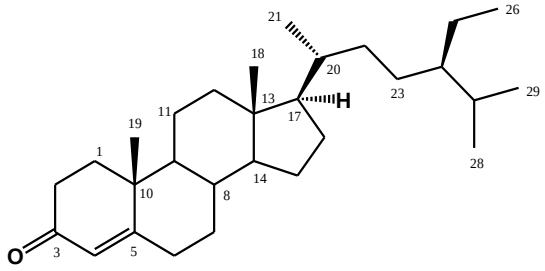

Figure 1. Chemical structure of stigma-4-en-3-on (**1**)

RESULTS AND DISCUSSION

Stigma-4-en-3-on(**1**) was obtained as a white crystals and dissolve in chlorofom.Liebermann-Burchard reaction of (Figure 1) gave a blue coloration, indicating a tetracyclic steroid. The molecular formula of (Figure 1) was established to be $C_{29}H_{48}O$ based on HRTOFMS spectra (*m/z* 413.3573 $[M+H]^+$ and NMR spectral data (Table 1), thus requiring six degrees of unsaturation. The IR spectrum showed strong absorption bands at 3150, 2960, 2870, and 1648 cm^{-1} which were due to an olefinic, aliphatic hydrocarbon and conjugated carbonyl groups. The ^{1}H NMR spectrum of (Figure 1) indicated the presence of two tertiery methyl at δ_H 0.64 and 1.11, three secondary methyl at δ_H 0.74 (3H, d, *J*=6.7 Hz), 0.77 (3H, d, *J*=2.64 Hz) and 0.84 (3H, d, *J*=6.54 Hz), one primary methyl at δ_H 0.77 (3H, d, *J*=6.5 Hz), one olefinic proton at δ_H 5.65 (1H, s) and remaining sp^3 aliphatic protons at δ_H 0.85-2.32 ppm, indicating the presence of steroidal skeleton in (Figure 1).

The ^{13}C-NMR spectrum showed twenty nine carbon resonances, which were classified by their chemical shifts, DEPT and the HMQC spectra as one carbonyl, two tertiary methyl, three secondary methyls, one primary methyl, one sp^2methine, one sp^2 quartenary carbon, eleven sp^3 methylene, seven sp^3 methines, and two sp^3 quartenary carbons. These functionalities accounted for two out of the total six degrees of unsaturation. The remaining four degrees of unsaturation were consistent with stigmastan

steroidstructure(Cayme and Ragasa, 2004; Phongmaykin et al., 2008). In order to clarify the position of functional group in structure of (Figure 1), HMBC and COSY experiments were carried out and the results was shown in Figure 2. The ^{1}H–^{1}H COSY spectrum of **1** showed correlations in C1–C2, C6–C7–C8–C9–C11–C12, C15–C16–C17 and C20-C21-C23-C24-C-25, supporting the presence of stigmastan steroidal structure.HMBC correlations between proton at H-2 (δ_H2.35) and H-4 (δ_H 5.65) to C-3 (δ_C199.7) and C-5 (δ_C 171.7), indicated that an α,β-unsaturated carbonyl group was located at C-3, C-4 and C-5, respectively. A methyl signal at δ_H 1.11 was correlated to C-1 (δ_C35.6), C-10 (δ_C 38.6), and C-9 (δ_C 52.8), whereas the another tertiary methyl at δ_H 0.64 was correlated to C-12 (δ_C 39.6), C-13 (δ_C 42.4) and C-17 (δ_C 56.1), indicated that two tertiary methyls were located at C-18 and C-19, respectively. A secondary methyl at δ_H 0.74 was correlated to C-17 (δ_C 56.1) and C-20 (δ_C 36.1), indicate that one of secondary methyl was located at C-21. Methyl signals at δ_H1.18 and 0.77 were correlated to C-27 (δ_C29.8), indicated that a *gem*-dimethyl group was located at C-27. A secondary methyl at δ_H0.81 was correlated to C-25 (δ_C 29.2) and C-24 (δ_C 45.8), indicated that a ethyl group was located at C-24. The stereochemistry of (Figure 1) was determined by NOESY experiments(Figure 3). The NOESY cross-peaks observed between H-2 and CH_3-10, and between H-4, H-6 and H-7, indicatedβ-configuration of methyl group at C-10. NOESY cross-peaks of H-16/H-17/H-20 indicated β-configuration of CH_3-18. This configuration was consistent of stigmastan steroid structure (Kolak et al., 2005).

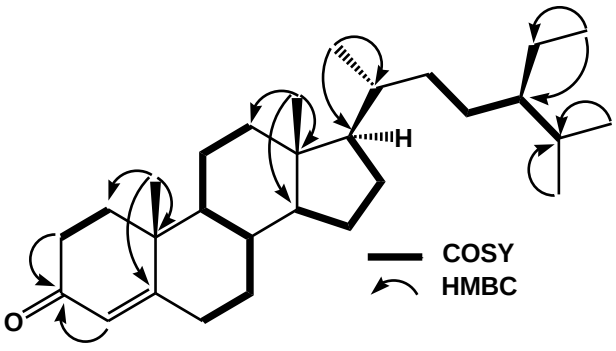

Figure 2. Selected HMBC and COSY correlations of stigmast-4-en-3-one(**1**)

Table 1. NMR data for compound 1 (500 MHz for ^{1}H and 125 MHz for ^{13}C)

Position of C	δ_H [ΣH, mult. *J* (Hz)]	δ_C (mult.)
1	1.62 (1H, m)	35.64 (t)
	1.96 (1H, m)	
2	2.29 (1H, m)	34.00 (t)
	2.35 (1H, m)	
3	-	199.7 (s)
4	5.55 (1H, s)	123.7 (d)
5	-	171.7 (s)
6	2.21 (1H, m)	32.9 (t)
	2.32 (1H, m)	
7	0.96 (1H, m)	32.1 (t)
	1.77 (1H, m)	
8	1.44 (1H, m)	35.7 (d)
9	0.85 (1H, m)	53.8 (d)
10	-	38.6 (s)
11	1.22 (1H, m)	21.0 (t)
	1.78 (1H, m)	
12	1.10 (1H, m)	39.6 (t)
	1.97 (1H, m)	
13	-	42.4 (s)
14	0.94 (1H, m)	55.8 (d)
15	1.38 (1H, m)	24.2 (t)
	1.54 (1H, m)	
16	1.22 (1H, m)	28.2 (t)
	1.78 (1H, m)	
17	1.05 (1H, m)	24.2 (d)
18	0.64 (3H, s)	11.9 (q)
19	1.11 (3H, s)	17.4 (q)
20	1.28 (1H, m)	36.1 (d)
21	0.74 (1H, d, 6.7)	18.7 (d)
22	0.94 (1H, m)	33.9 (t)
	1.25 (1H, m)	
23	1.08 (1H, m)	26.1 (t)
	1.09 (1H, m)	
24	0.85 (1H, m)	45.8 (d)
25	1.60 (2H, t, 6.5)	29.2 (t)
26	0.84 (3H, d, 6.5)	19.0 (q)
27	1.18 (1H, m)	23.1 (d)
28	0.79 (3H, d, 2.6))	19.8 (q)
29	0.77 (3H, d, 2.6)	11.9 (q)

A detailed comparison of spectral data of 1 with those of previously reported, stigmast-4-en-3-one (Kolak et al., 2005), revealed that both compounds showed very similar, consequently

compound 1 was identified as a stigmast-4-en-3-one and was shown for the first time in this species.

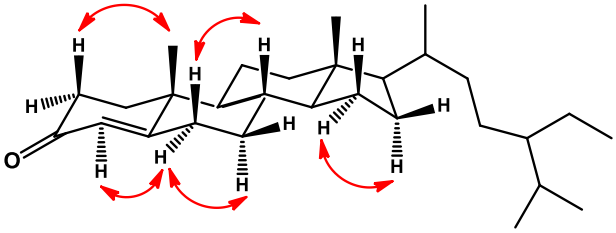

Figure 3. NOESY correlations of stigmast-4-en-3-one (**1**)

CONCLUSION

A stigmastane steroid, stigmast-4-en-3-one (1) has been isolated from the bark of *Chisocheton lansiocarpus* (Meliaceae). Its chemical structure was identified on the basis of spectroscopic datas and by comparison with previous data reported previously. Stigmast-4-en-3-one (1) was the first time isolated from *Chisocheton lansiocarpus*and give more information the occurance the stigmastane steroid in genus *Chisocheton*.

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