Stigmast-5(6)-en-3β-ol from the Bark of *Chisocheton lasiocarpus* (Meliaceae)

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Abstract
Chisocheton lasiocarpus is one of species from Meliaceae family. Investigation on secondary metabolites of C. lasiocarpus, grown in Indonesia, has not been reported. In this study, a stigmast-5(6)-en-3β-ol compound has been successfully isolated from the bark of C. lasiocarpus by using methods of extraction, partition, and chromatography. Methanolic extract of C. lasiocarpus was partitioned successively to give n-hexane, ethyl acetate and n-butanol extracts.

The n-hexane extract was separated and purified by chromatography methods to obtain the pure isolate. The chemical structure of stigmast-5(6)-en-3β-ol was elucidated based on spectroscopic data and by comparison of spectral data obtained previously.

Keywords: *Chisocheton lasiocarpus*, stigmast-5(6)-en-3β-ol compound, steroid, stigmastan, Meliaceae.

Introduction

The Meliaceae plant has 51 genus and 550 species that are widely distributed in subtropical and tropical countries. The family plants have been known as plant-producing secondary metabolites that have diverse biological activities such as antimalaria[1,2,3], cytoxic[4,5], insecticides, antivirals, antioxidants, anticancer[6], antiprotozoa[7], antibacteria, antimicrobe, anti-inflammatory[8] and antifeedant[9,10].

The *Chisocheton* genus, a member of the Meliaceae family, contains approximately 50 species that are mainly distributed in India, Thailand, Malaysia and Indonesia.[11-13] The genus is a subtropical and tropical plant family, widely known for its insecticidal limonoid constituents[14]. Previous phytochemical studies on *Chisocheton* species have yielded a number of interesting compounds including limonoids,[15-17] antifungal meliacin-type compound[18], dammarane triterpenoids[19] and spermidine alkaloids[20].

As part of our studies on compounds from Indonesia Meliaceae plants[21,22], we carried out a study on *Chisocheton lasiocarpus*. *C. lasiocarpus* is widely dispersed in the Solomon Islands, Malesia, Maluku (Seram), Papua New Guinea. The bark of *C. lasiocarpus*[21] was studied in this current research.

Tzouros et al[22] reported the presence of new secondary metabolites, two spermidin alkaloid with open rings, from *C. weinlandii*, which are synonymous with *C. lasiocarpus*.

In this communication, we describe the isolation and structural elucidation of stigmast-5(6)-en-3β-ol from the bark of *C. lasiocarpus*. Their structures were elucidated by spectroscopic methods including IR, 1D-NMR (^{1}H, and ^{13}C) and ^{1}H-^{1}H COSY.

Material and Methods

Material: The bark *C. lasiocarpus* was collected in Bogor Botanical Garden, Bogor, West Java Province, Indonesia in April 2017. The plant was identified by the Staff of the Bogoriense Herbarium, Bogor, Indonesia and was deposited at the herbarium.Melting points were measured on a Mettler Toledo micro melting point apparatus and are uncorrected. The IR spectra were recorded on a Perkin-Elmer spectrum-100 FT-IR in KBr. ^{1}H- and ^{13}C-NMR spectra were obtained with a JEOL JNM A-500 spectrometer using TMS as internal standard. Chromatographic separations were carried out on silica gel 60 (Merck). TLC glass plates were precoated with silica gel GF254 (Merck, 0.25 mm) and detection was achieved under UV light at λ 254 and 367 nm and by spraying with 10% H_2SO_4 in ethanol followed by heating.

Methods

Isolation and purification: The bark of *C. lasiocarpus* (4.1 kg) was extracted with methanol over the period of 3 days at room temperature. The extract was filtered and concentrate under reduced pressure to provide the viscous concentrated of MeOH extract (209 g). The crude extract was first suspended in H_2O and then partitioned with *n*-hexane, EtOAc and *n*-butanol successively. Evaporation resulted in crude extract of *n*-hexane (10.5 g), EtOAc (20.0 g), and *n*-BuOH (50.0 g) respectively. A portion of *n*-hexane soluble fraction (10.5 g) was subjected to vacuum liquid chromatography using gradient elution of *n*-hexane-EtOAc (10:0-0:10) to afford six fractions (A01-A6). Fraction A01 (1.71 g) was subjected to silica gel column chromatography using eluent of *n*-hexane/dichloromethane (10:0-0:10) as eluting solvents to afford 11 fractions (1A-1K). Fraction 1K (0,09 g) was recrystallized with *n*-hexane to give the isolate (20.0 mg).

Stigmast-5(6)-en-3β-ol: Colorless crystals; m.p. 131-133°C; IR (KBr) cm^{-1} 3420, 1755, 1740, 1690, 1610. ^{1}H-NMR ($CDCl_3$, 500 MHz), (table 1); ^{13}C-NMR ($CDCl_3$, 125 MHz), (table 1).

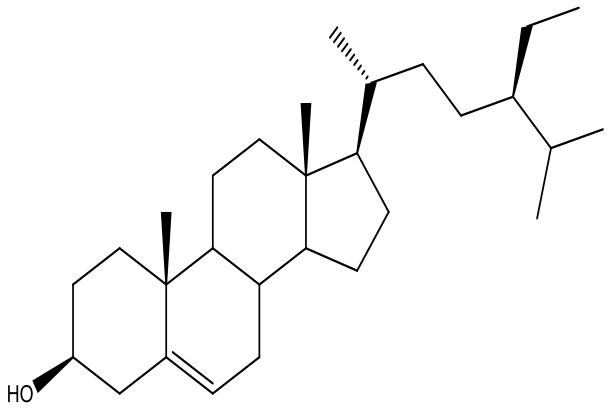
Results and Discussion

Stigmast-5(6)-en-3β-ol was isolated as colorless crystals NMR analysis indicates molecular formula of $C_{29}H_{50}O$

which requires five degrees of unsaturation. The IR spectrum of compound showed the presence of hydroxyl group (υ_{max} 3420 cm^{-1}), olefinic (υ_{max} 1654 cm^{-1}), eter (υ_{max} 1054 cm^{-1}), and gem dimethyl (υ_{max} 1461- 1376 cm^{-1}) functionalities. The ^{1}H-NMR spectrum (Table 1) showed the presence of two tertiary methyl groups (δ_H 0.72, 0.82, each 3H, s), three secondary methyl groups (δ_H 0.84, 0.86 and 0.97, each 3H, s), one primary methyl group (δ_H 0.88), one oxygenated proton (δ_H 3.38[1H, q, 4.5,5.2]) and one olifenic group (δ_H 5.30 [1H, d, J = 5.15 Hz]). The observed proton signal data suggested the presence of stigmast steroid skeleton.

A total of 29 carbon resonances was observed in the ^{13}C-NMR spectrum (Table 1). These were assigned by ^{1}H-^{1}H COSY experiments, revealing one sp^3 oxygenated carbon (δ_C 74.8), two sp^2 carbons (δ_C 122.8, 141.0), six methyls, eleven sp^3 methylenes, one sp^2 methine (δ_C 122.8), two sp^3 carbon quartenary (δ_C 37.3, 42.1) and one sp^2 carbon quartenary. These functionalities accounted for one out of the total five degrees of unsaturation.

The remaining four degrees of unsaturation were consistent with a stigmastan structure[23]. The obtained isolate was identified as stigmast-5(6)-en-3β-ol, on the basis of NMR, as well as by comparison of their spectral data.[24]



Stigmast-5(6)-en-3β-ol

Conclusion

The stigmastan steroid compound was successfully isolated from the bark of *C. lasiocarpus* and obtained as colorless crystals. The chemical structure of the stigmast-5(6)-en-3β-ol compound was determined based on spectroscopic data obtained and comparison previously reported.

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Table 1
NMR data stigmast-5(6)-en-3β-ol

Posisi	^{1}H- NMR	^{13}C-NMR
	δ_H (Integral, mult, J=Hz) δc (mult.)	**δ_C (Integral, mult, J=Hz) δc (mult.)**
1	1.59 (1H, m)	37.3 (t)
	1.56 (1H, m)	-
2	1.53 (1H, d, 11.05)	30.6 (t)
	1.77 (1H, d, 11.05)	-
3	3.38 (1H, q, 4.5, 5.2)	74.8 (d)
4	2.21 (2H, d, 2.55)	39.7 (d)
5	-	141.0 (s)
6	5.30 (1H, d, 5.15)	122.8 (d)
7	1.97 (2H, m)	33.9 (d)
8	1.42 (1H, m)	32.0 (d)
9	0.92 (1H, m)	49.4 (d)
10	-	37.3 (s)
11	1.56 (2H, m)	21.6 (t)
12	1.85 (2H, m)	39.6 (t)
13	-	42.1 (s)
14	1.08 (1H, m)	56.3 (d)
15	1.15 (2H, m)	25.9 (t)
16	1.16 (2H, m)	26.3 (t)
17	1.05 (1H, m)	57.5 (d)
18	0.72 (3H, s)	12.2 (q)
19	0.82 (3H, s)	19.3 (q)
20	1.36 (1H, m)	37.3 (d)
21	0.97 (3H, d, 5.8)	19.3 (q)
22	1.22 (2H, m)	34.9 (t)
23	1.68 (2H, m)	25.9 (t)
24	1.46 (1H, m)	47.4 (d)
25	1.56 (1H, m)	33.3 (d)
26	0.86 (3H, d, 6.2)	14.1 (q)
27	0.84 (3H, d, 6.2)	20.3 (q)
28	1.32 (2H, m)	24.0 (t)
29	0.88 (3H, t, 1.9)	14.4 (q)

* Measurements in CD_3OD for ^{1}H-NMR at 600 MHz and ^{13}C-NMR at 150 MHz

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