Jurnal Sains dan Matematik Vol.5 No.1 (2013) 28-34
ISSN 1985-7918

Isolation of Stigmasterol from the Bark of *Kopsia singapurensis* Ridl.

Pengasingan Stigmasterol dari Kulit Kayu *Kopsia singapurensis* Ridl.

Tan Siow Ping[1], Mohd Azlan Nafiah[1], Lee Yean Shan[1], Khalijah Awang[2] & *Kartini Ahmad[1]
[1]Department of Chemistry, Faculty of Science and Mathematics, University Pendidikan Sultan Idris, 35900 Tanjung Malim, Perak.
[2]Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur.
e-mail: *kartini@fsmt.upsi.edu.my

Abstract

General phytochemical screening on the bark of *Kopsia singapurensis* Ridl. (Apocynaceae) revealed the presence of steroids, terpenes and alkaloids. Stigmasterol **1** was isolated from the hexane crude extract of this species. The structure of this compound was elucidated based on spectroscopic analysis such as ultra-violet (UV), infrared (IR), gas chromatography - mass spectroscopy (GC-MS), nuclear magnetic resonance (NMR), and involving also comparison with data from the literature. This compound has never been reported from this species.

Keywords Phytochemical, *Kopsia singapurensis*, Apocynaceae, steroids, stigmasterol

Abstrak

Saringan fitokimia umum ke atas batang pokok *Kopsia singapurensis* Ridl. (Apocynaceae) telah didapati mengandungi steroid, terpena, dan alkaloid. Stigmasterol **1** telah diasingkan daripada ekstrak mentah heksana spesis ini. Struktur sebatian dikenalpasti melalui spektroskopi analisis seperti ultra lembayung (UL), inframerah (IM), kromatografi gas - spektroskopi jisim (KG-SJ), resonans magnet nuklear (RMN) dan juga melibatkan perbandingan dengan kajian-kajian lepas. Sebatian ini tidak pernah dilaporkan daripada spesis ini.

Kata kunci Fitokimia, *Kopsia singapurensis*, Apocynaceae, steroid, stigmasterol

Introduction

Despite its rapid urban development, Malaysia is still remaining wealthy in her medicinal plants from its tropical rain forests. 2,000 species from 14,500 flowering plants have been reported to contain medicinal properties and many have been scientifically proven (Rizwana *et al*., 2010).

Genus *Kopsia* is placed in the tribe Rauvolfieae of the subfamily Rauvolfioideae (Middleton, 2004). Twenty four species of the plants of this genus are recognized and widely distributed over Southeast Asia, India and China (The Plat List, 2010). About 18 species are distributed in Malaysia (Awang *et al*., 2008; Kam, Yoganathan, & Chuah,

1997). This genus is known to produce a large number of biologically active indole alkaloids possessing interesting skeletons. Various medicinal uses have been reported; the roots of *K. larutensis* King & Gamble, *K. macrophylla* Hook f., *K. singapurensis* Ridl. and *K. pauciflora* Hook f. are used to treat poulticing ulcerated noses in tertiary syphilis (Burkill, 1966) while *K. officinalis* Tsiang & Li is used in Chinese traditional medicine for rheumatoid arthritis, dropsy and tonsillitis (Zhou *et al*., 2006).

Kopsia singapurensis Ridl., also known as white kopsia, is the only endemic species found in Malaysia and Singapore (Middleton, 2004). Previously, wide range of chemical compounds including alkaloids, sterols and terpenoids had been isolated from various *Kopsia* plants, particularly those indigenous to Malaysia, has led to the isolation of more than 180 alkaloids (Kam & Lim, 2008). However, there is no report on the isolation of steroids from this species in the literature to date.

The purpose of this study is to isolate and identify the chemical compounds from the bark of *K. singapurensis.* We will only describe the isolation and structural elucidation of a known compound namely stigmasterol **1** in the present paper.

Materials and methods

General Methods

Nuclear Magnetic Resonance spectra (NMR) were recorded using Bruker (600 MHz). Deuterated chloroform ($CDCl_3$) was used as the NMR solvent. Chemical shifts (δ) were reported in ppm and coupling constants (*J*) in Hz. Mass spectra (MS) were determined by using the GC-mass spectrometry (GC-MS Agilent 5975 Series). The ultra violet spectrum (UV) were obtained in methanol on a Perkin Elmer uv-visible spectrophotometer and the wavelength of the spectrum was determined in the range of 200 to 1000 nm. The infrared spectrum (IR) were recorded on a Nicolet 6700 FTIR spectrophotometer, with CH_2Cl_2 as dilution solvent of the sample.

Column chromatography were prepared by using Silica Gel $60F_{254}$, 70-230 mesh ASTM and 230-400 mesh ASTM and Silica Gel $60F_{254}$ containing Gypsum as stationary phase. Analytical thin layer chromatography (TLC) was performed on commercially precoated aluminium supported silica gel $60F_{254}$ TLC sheets.

Plant Material

The species selected for the current study is *Kopsia singapurensis* Ridl. (KL5334) which was collected from Kluang, Johor and identified by the phytochemical group, Chemistry Department, Faculty of Science, University of Malaya. The Voucher specimens were deposited at the Chemistry Department, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia and the Herbarium of the Forest Research Institute, Kepong, Malaysia.

Extraction and Isolation

Extraction of the bark of *Kopsia singapurensis* (3.3 kg) was carried out by exhaustive Soxhlet extraction. Dried grounded bark of the plant were first defatted by using hexane for

Jurnal Sains dan Matematik Vol.5 No.1 (2013) 28-34
ISSN 1985-7918

17 hours at room temperature. Then, the filtrate was dried by using the rotary evaporator and give 58.0 g of hexane crude extract. For preliminary fractionation, the hexane crude extract (30.0 g) was chromatographed over silica gel and eluted with a mixture of hexane, dichloromethane and methanol by increasing its polarity to give eight fractions. Each fraction was monitored by using Aluminium supported TLC plate and 5% sulphuric acid was used as spraying agents. Fraction 6 (1.94 g) was rechromatographed on a silica gel column to give 30.9 mg of stigmasterol [**1**, R_f = 4.1 cm in CH_2Cl_2:MeOH (99:1)].

Spectroscopic Characterization

Different spectroscopic methods were used to elucidate the structure of isolated compound **1**. Among the spectroscopic techniques UV, IR, ^{1}H-NMR, ^{13}C-NMR and GC-MS were carried out. **Stigmasterol** ($C_{29}H_{48}O$) **1**: Colourless needles; UV (CH_2Cl_2) λ_{max} 276.8 (4.65) nm; IR_{vmax} (CH_2Cl_2): 3055 (O-H), 1711 (C=C), 1263 (C-O) cm^{-1}. GC-MS *m/z* 412 [M$^+$], 394, 351, 314, 300, 271, 229, 213, 55. ^{1}H and ^{13}C NMR see Table 1.

Results and discussion

Compound **1** was obtained as colourless needles (m.p. 151.0 – 152.0 °C) (Prachayasittikul *et al*., 2009). The compound was found to be a C-5 unsaturated modified tetracyclic triterpene, which showed only 29 carbons instead of 30 carbons for tetracyclic triterpenes. This was further supported by the UV spectrum which showed maxima absorption at 276.8 nm (log ε = 4.65) (Burnouf-Radosevich *et al*., 1984).The IR spectrum displayed stretching band for O-H (3300 cm^{-1}), olefin (1711 cm^{-1}) and C-O (1263 cm^{-1}). Its molecular formula of $C_{29}H_{48}O$ was established from GC-MS which give a [M]$^+$ ion at *m/z* 412.

The ^{1}H NMR spectrum (Table 1 and Figure 1) of **1** displayed signals for six methyls at δ 0.68 (Me-18, *s*), δ 1.00 (Me-19, *s*), δ 1.49 (Me-21, *d*, *J* = 6.4 Hz), δ 0.85 (Me-26, *d*, *J* = 5.4 Hz), δ 0.79 (Me-27, *d*, *J* = 5.6 Hz) and δ 0.84 (Me-29, *t*, *J* = 6.8 Hz). The proton of H-3 appeared as multiplet at δ 3.52 corresponding to hydroxyl methine proton. The typical signal for the olefinic proton (H-6) of the steroidal skeleton was evident from a multiplet at δ 5.35 integrating for one proton. Another two olefinic protons appeared as characteristics downfield signals at δ 5.14 (H-22) and δ 5.00 (H-23). Each of the signal was observed as doublet of doublets (*dd*, *J* = 15.1 and 8.7 Hz) which indicate coupling with the neighbouring olefinic and methine protons in *trans*-orientation (Sharma *et al*., 2010).

Table 1 ^{1}H NMR [600 MHz, δ_H (*J*, Hz)] and ^{13}C NMR [100 MHz, δ_C] of **1** in $CDCl_3$

Position	δ_H (*J* Hz), ppm	δ_C, ppm	DEPT	Stigmasterol, [100MHz, $CDCl_3$] (Jamal *et al*., 2009)
1	1.07 (*m*)	37.3	CH_2	37.4
	1.84 (*m*)			
2	1.51 (*m*)	31.7	CH_2	28.4
	1.83 (*m*)			
3	3.52 (*m*)	71.8	CH	72.0
4	2.23 (*m*)	42.3	CH_2	37.5
	2.28 (*m*)			
5		140.8	qC	140.9
6	5.35 (*m*)	121.7	CH	121.9
7	1.46 (*m*)	31.9	CH_2	31.9
	1.96 (*m*)			
8	1.58 (*m*)	31.9	CH	32.1
9	0.93 (*m*)	50.2	CH	50.3
10		36.5	qC	36.7
11	1.02 (*m*)	21.1	CH_2	21.3
	1.49 (*m*)			
12	1.17 (*m*)	39.7	CH_2	39.8
	2.01 (*m*)			
13		42.2	qC	42.4
14	0.99 (*m*)	56.9	CH	57.0
15	1.06 (*m*)	24.4	CH_2	24.5
	1.56 (*m*)			
16	1.25 (*m*)	28.9	CH_2	29.2
	1.70 (*m*)			
17	1.14 (*m*)	56.0	CH	56.1
18	0.68 (*s*)	11.8	CH_3	12.1
19	1.00 (*s*)	19.4	CH_3	19.1
20	2.03 (*m*)	40.5	CH	40.7
21	1.49 (*d*, 6.4 Hz)	21.2	CH_3	21.4
22	5.14 (*dd*, 15.1 Hz and 8.7 Hz)	138.3	CH	138.5
23	5.00 (*dd*, 15.1 Hz and 8.7 Hz)	129.7	CH	129.5
24	1.54 (*m*)	51.2	CH	51.4
25	1.45 (*m*)	50.1	CH	46.0
26	0.85 (*d*, 5.4 Hz)	21.2	CH_3	19.6
27	0.79 (*d*, 5.6 Hz)	19.0	CH_3	20.0
28	1.01 (*m*)	24.3	CH_2	25.6
29	0.84 (*t*, 6.8 Hz)	12.1	CH_3	12.2

Jurnal Sains dan Matematik Vol.5 No.1 (2013) 28-34
ISSN 1985-7918

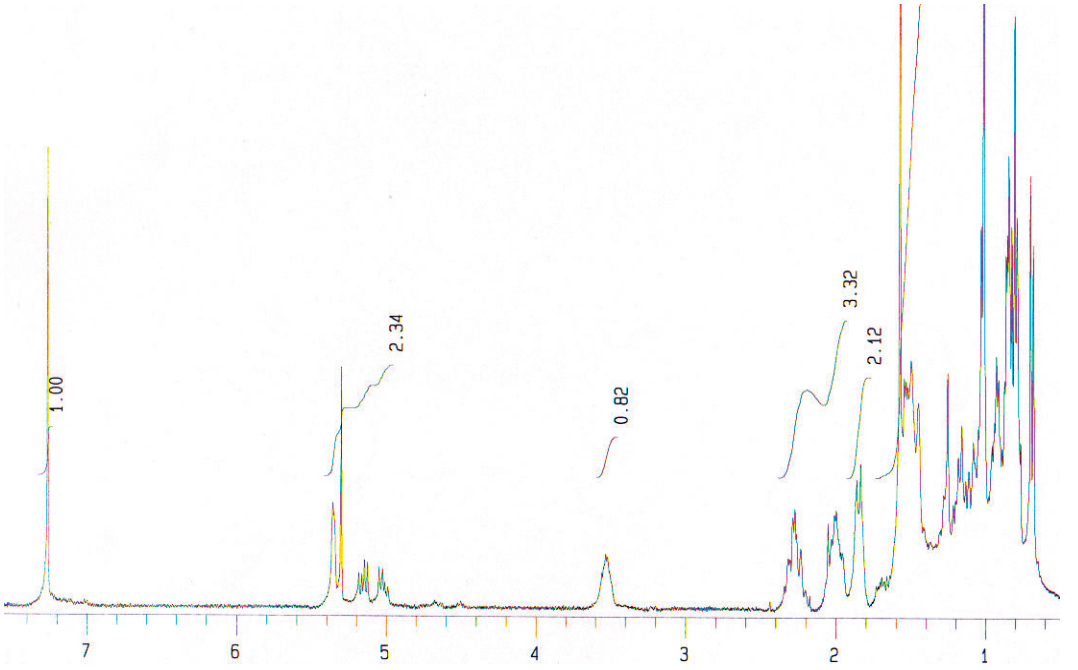


Figure 1 ^{1}H NMR Spectrum of **1`**

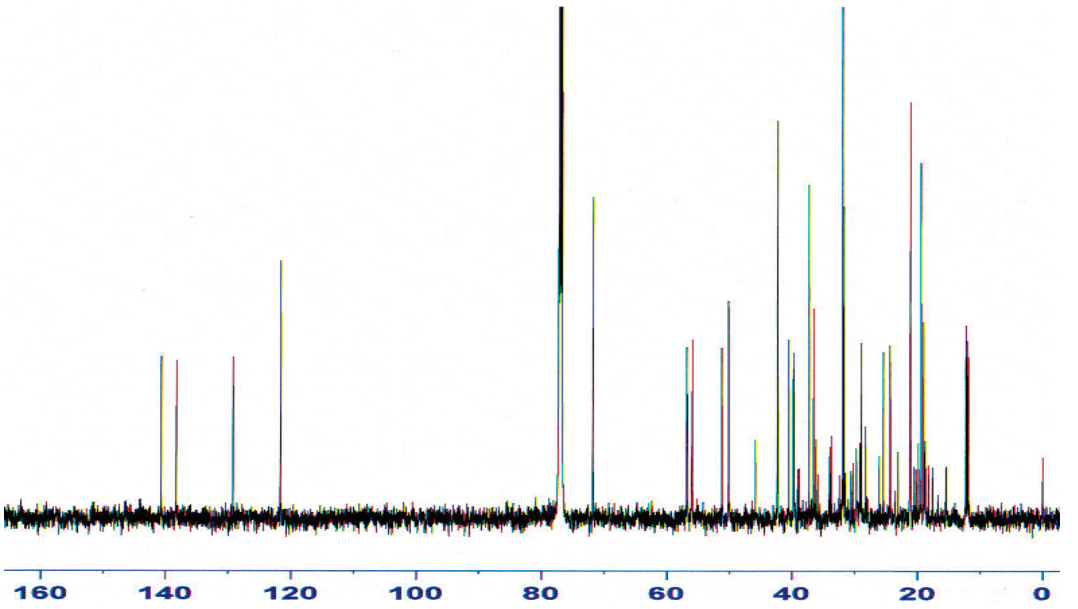
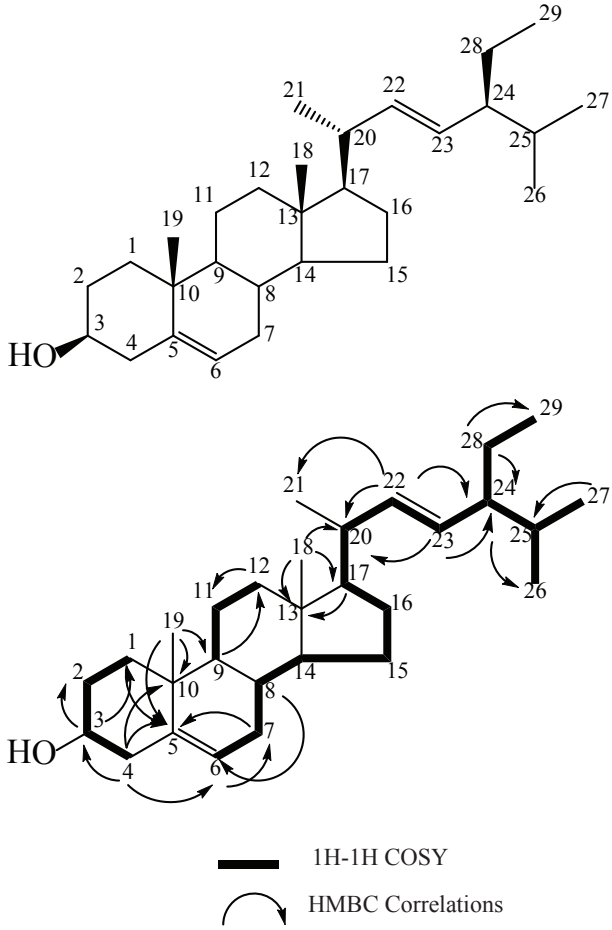


Figure 2 ^{13}C NMR Spectrum of **1**

The ^{13}C NMR spectrum (Table 1 and Figure 2) of **1** showed a total of 29 carbon atoms. Among the 29 carbons, the ^{13}C data indicated the presence of three quaternary carbons: δ

140.8 (C-5), δ 36.5 (C-10), δ 42.2 (C-13); eleven methine carbons: δ 71.8 (C-3), δ 121.7 (C-6), δ 31.9 (C-8), δ 50.2 (C-9), δ 56.9 (C-14), δ 56.0 (C-17), δ 40.5 (C-20), δ 138.3 (C-22), δ 129.7 (C-23), δ 51.2 (C-24), δ 50.1 (C-25); nine methylene carbons: δ 37.3 (C-1), δ 31.7 (C-2), δ 42.3 (C-4), δ 31.9 (C-7), δ 21.1 (C-11), δ 39.7 (C-12), δ 24.4 (C-15), δ 28.9 (C-16), δ 24.3 (C-28); and six methyl carbons: δ 11.8 (C-18), δ 19.4 (C-19), δ 21.2 (C-21), δ 21.2 (C-26), δ 19.0 (C-27), δ 12.1 (C-29). There are four olefinic carbon signals at δ 140.8 (C-5), δ 121.7 (C-6), δ 138.3 (C-22) and δ 129.7 (C-23).

The assignment were further supported by ^{1}H-^{1}H COSY that suggested the following fragments: C-1 – C-4, C-6 – C-8 – C-14 – C-17 – C-20 – C-22 – C-26, C-25 – C-27, C-24 – C-28 – C-29 and C-9 – C-11 – C-12. The HMBC correlations showed unambiguous assignment of the protons and carbons. Its showed cross signals from H-2 to C-3 and C-10; H-4 to C-5, C-6 and C-10; H-6 to C-4 and C-7; H-18 to C-13, C-17 and C-20; H-22 to C20, C-21, C-23 and C-24; H-23 to C-20 and C-24. There are several other correlations were observed which also confirmed the attribution of the ^{13}C signals of compound **1**. The compound was assigned as Stigmasterol based on the spectral data and comparison with the literature values (Jamal *et al*., 2009).



Jurnal Sains dan Matematik Vol.5 No.1 (2013) 28-34
ISSN 1985-7918

Conclusion

The present study was aimed to investigate the chemical constituents of *Kopsia singapurensis* Ridl. (KL 5334) collected from Kluang, Johor. Stigmasterol **1**, a known steroid, was isolated from the bark of *Kopsia singapurensis*. The spectral data of the compound was also compared with those in the literature.

Acknowledgement

We gratefully acknowledged generous support of Universiti Pendidikan Sultan Idris and the excellent technical assistance from University of Malaya.

References

Awang, K., Ahmad, K., Thomas, N. F., Hirasawa, Y., Takeya, K., Mukhtar, M. R., Mohamad, K. & Morita, H. (2008). Singaporentine A: A New Indole Alkaloid from *Kopsia singapurensis* Ridl. *Heterocycles,* 75(12), 3051-3056.

Burkill, H.I. (1966). *A Dictionary of the Economic Products of the Malay Peninsula*, Ministry of Agriculture and Cooperatives, Kuala Lumpur, Malaysia:

Burnouf-Radosevich, M. & Delfel, N.E. (1984). High-Performance Liquid Chromatography of Oleanane-Type Triterpenes. *Journal of Chromatography*, 292, 403–409.

Jamal, A.K., Yaacob, W.A. & Laily, B.D. (2009). A Chemical Study on *Phyllanthus reticulatus*. *Journal of Physical Sciences*, 19(2), 45–50.

Kam T.S. & Lim K.H. (2008). *The Alkaloids*. Cordell G. A., Ed. Academic Press, Amsterdam, Vol. 66, 1–105.

Kam, T. S., Yoganathan, K. & Chuah, C. H. (1997). Four Heptacyclic Indoles from *Kopsia teoi*. *Phytochemistry,* 45(3), 623–625.

Middleton, D. J. (2004). A Revision of *Kopsia* (Apocynaceae: Rauvolfioideae). *Harvard Paper in Botany,* 9(1), 89–142.

Prachayasittikul, S. , Suphapong, S., Worachartcheewan, A., Lawung, R., Ruchirawat, S. & Prachayasittikul, V. (2009). Bioactive Metabolites from *Spilanthes acmella* Murr. *Molecules,* 14*,* 850–867.

Rizwana, J. N., Nazlina, L., Mohd Razehar, A. R., Siti Noraziah, A. Z. & Ling, S. A. (2010). A Survey on Phytochemical and Bioactivity of Plant Extracts from Malaysian Forest Reserves. *Journal of Medical Plants Research.* 4(3), 203–210.

Sharma, S., Vijayvergia, R. & Singh, T., (2010). Extraction and Identification of Pentacyclic Compound β-amyrin (Terpenoid). *Archives of Applied Science Research*, 2 (2), 124–126.

The Plant List (2010). Version 1. Retrieved from http://www/theplantlist.org/.

Zhou, H., He, H.P, Kong, N.C., Wang, Y.H., Liu, X.D. & Hao, X.J. (2006). Three new indole alkaloids from the leaves of *Kopsia officinalis. Helvetica Chimica Acta*, 89, 515.