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SINGAPORENTINE A: A NEW INDOLE ALKALOID FROM *KOPSIA SINGAPURENSIS* RIDL.

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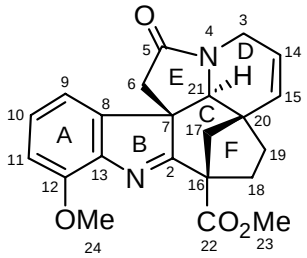
Abstract – Study on chemical constituents from the leaves of *Kopsia singapurensis* (Apocynaceae) yielded a new *indole* alkaloid, singaporentine A (**1**) together with two known alkaloids, kopsifoline A (**2**) and kopsininic acid (**3**). The structure of **1** was elucidated by combination of various spectroscopic methods such as MS, UV, IR, 1D and 2D NMR. Singaporentine A exhibited cytotoxic activity against P388 murine leukemia cells.

INTRODUCTION

The genus *Kopsia* (Apocynaceae) comprises of 30 species which is native to China, India and Southeast Asia.[1-7] This genus is known to produce a large number of biologically active indole alkaloids possessing interesting skeletons.[1-7] 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[8] while *K. officinalis* Tsiang & Li is used in Chinese traditional medicine for rheumatoid arthritis, dropsy and tonsillitis.[9] Previous chemical investigation on *Kopsia singapurensis* afforded several skeletal types of indole alkaloids such as kopsingine, kopsaporine, kopsingarine,[10] singapurensines A-D[11] and kopsiloscine A-F[12] (aspidofractinine type), rhazinilam and rhazinal[12] (aspidosperma type), vincophylline[12] (vincorine type), 16-epideacetyakuammiline[12] (akuammiline type), mersinaline and mersirachine[13] (mersinine type). Our continuing study on the leaves of *Kopsia singapurensis* Ridl., have afforded three indole alkaloids; singaporentine A **(1)** , kopsifoline A

(2)[14,15] and kopsininic acid **(3)**.[16] The former, singaporentine A **(1)**, is new and its structure was elucidated by using spectroscopic techniques such as 1D and 2D NMR, IR, UV and MS.



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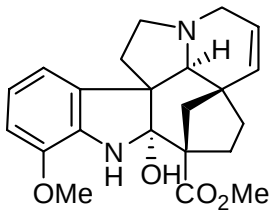

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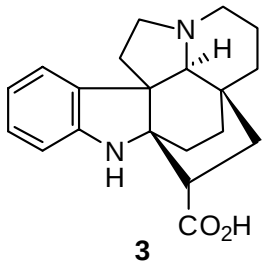

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RESULTS AND DISCUSSION

Singaporentine A **(1)** was isolated from dichloromethane extract as a light yellowish oil, with $[\alpha]_D^{26}$ -23° (*c* = 0.3, MeOH). The UV spectrum revealed the maximum absorptions at 232, 253 and 310 nm indicating the presence of an indolenine chromophore.[13,14] The IR spectrum showed the band at 1738 and 1720 cm^{-1} which were assigned to carbonyl functional groups for methyl ester and lactam,[17] respectively. The HRESIMS of **1** gives a pseudomolecular ion peak at m/z 379 corresponding to the molecular formula of $C_{22}H_{22}N_2O_4$ (*m/z* 379.1659 $[M + H]^+$; calc. 379.1658).

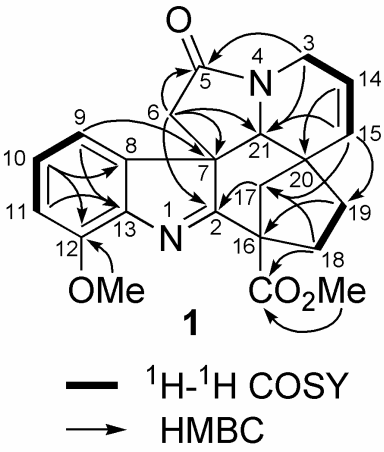

Figure 1. Selected 2D NMR correlations for singaporentine A (**1**).

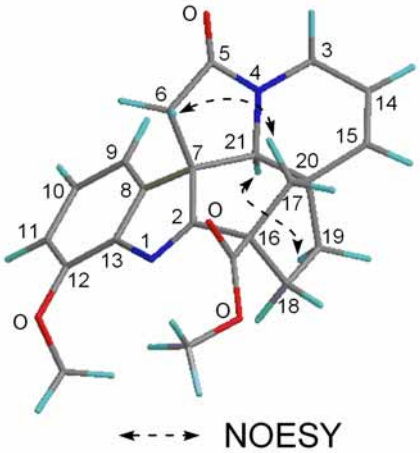

Figure 2. Selected NOESY correlations and relative stereochemistry for singaporentine A (**1**).

^1H and ^{13}C NMR data (Table 1) indicated the presence of five sp^2 methines, six sp^2 quaternary carbons, five sp^3 methylenes, one sp^3 methine, three sp^3 quaternary carbons and two methyl groups. Two of the sp^2 quaternary carbons (δ_C 183.3, 142.3) were attached to the nitrogen atom in the indolenine ring (N-1). One sp^3 methylene (δ_C 40.0), one sp^3 methine (δ_C 64.8) and one sp^2 quaternary carbon (δ_C 171.1) were attached to the other nitrogen atom (N-4).

The ^{1}H-^{1}H COSY spectrum of **1** suggested the following three fragments; C-3–C-15, C-9–C-11 and C-18–C-19. The HMBC spectrum showed correlation between C-18 and the isolated methylene protons of C-17 which resonated as a broad doublet (J=13.2 Hz) in the ^{1}H NMR spectrum. In addition, correlation signals were also observed for H_2-17 and C-2, C-16, C-18, C-19 and C-20 thus confirmed the five-membered ring nature of ring F and its connection to both ring C and D, respectively. The methoxy at C-12 was confirmed by the presence of cross-peaks between H_3-24 and C-12, and H-9 with C-7. The HMBC cross-peaks of H-3β to C-5 and C-21 indicated the connection among C-3, C-5 and C-21 through N-4. The carbonyl signal at δ_C 171.1 and the IR absorption of carbonyl group at 1720 cm^{-1} indicated the presence of the five-membered lactam ring (ring E).[17] The connection among C-15, C-17, C-19 and C-21 through C-20 was deduced from HMBC correlations of H-14 to C-20, and H-15 to C-17, C-19 and C-21. In addition, the connections between C-2, C-17, C-18 and C-22 through C-16 were elucidated by HMBC correlations of H-19β to C-16, H-18β to C-17 and C-22, and H-17α to C-2. These observations further confirmed that the six-membered ring C is fused to the five-membered ring F. The connectivity between the methoxy group (OMe-23) and C-22 was established from HMBC correlations between of H_3-23 and C-22. Thus, the gross structure of singaporentine A was deduced to be **1**.

The relative stereochemistry of **1** was established by NOESY correlations as shown in the computer-generated 3D drawing (Figure 2). NOESY correlations of H-6/H-17β and H-19α/H-21 indicated that singaporentine A possessed the same stereochemistry with kopsifoline E[15], previously isolated from the leaf of *K. fruticosa*. Therefore the relative stereochemistry of **1** is as depicted in Figure 2.

Singaporentine A belongs to a rare type of indole alkaloids. At present, only six alkaloids possess this peculiar skeleton; kopsifoline A-F from *K. fruticosa*.[14,15] This is the third report on the occurrence of this special alkaloid. Both singaporentine A **(1)** and kopsininic acid **(3)** showed *in vitro* cytotoxic activity against P388 cells[18] at IC_{50} 57μg/ml and IC_{50} 48μg/ml, respectively.

Table 1: ^{1}H NMR [400 MHz, δ_H (*J*, Hz)] and ^{13}C NMR [100 MHz, δ_C] of (**1**) in $CDCl_3$

Position	^{13}C	^{1}H (*J* Hz)
2	183.3	
3	40.0	α 3.62 *d* (18.0)
		β 4.60 *d* (18.0)
4	-	-
5	171.1	-
6	39.1	α 2.56 *d* (18.0)
		β 3.26 *d* (18.0)
7	55.3	-
8	146.7	-
9	112.8	6.93 *d* (8.0)

10	128.5	7.22 *t* (8.0)
11	111.5	6.90 *d* (8.0)
12	151.4	-
13	142.3	-
14	123.9	5.74 *d* (10.0)
15	132.4	5.63 *d* (10.0)
16	56.6	-
17	41.7	α 1.81 *d* (12.4)
		β 2.24 *d* (13.2)
18	39.6	α 2.37 *m*
		β 2.42 *m*
19	33.0	α 1.21 *m*
		β 1.74 *m*
20	45.0	-
21	64.8	3.49 *s*
CO_2Me	52.7	3.73 *s*
C=O	171.2	-
12-OMe	55.5	3.97 *s*

EXPERIMENTAL

General.

Spectra were recorded on the following instruments: uv, Shimadzu UV-250 uv-visible spectrophotometer; ir, Perkin Elmer 1600; optical rotations at 25^o were taken on Jasco DIP-1000 Digital polarimeter. UV and optical rotation were recorded in MeOH. LC-EIMS, Waters Micromass ZQ; nmr, Bruker AV 400 MHz and JEOL ECA 400 MHz.

Plant Material.

The leaves of *Kopsia singapurensis* were collected in Kluang, Johor, Malaysia 2007. Identification was made by Mr. Teo Leong Eng, University of Malaya. Voucher specimens (KL 5334) were deposited at Herbarium of the Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia and at the Herbarium of the Forest Research Institute, Kepong, Malaysia.

Extraction and Isolation.

The dried leaves (2 kg) of *Kopsia singapurensis* were ground and extracted exhaustively with hexane followed by CH_2Cl_2 by soxhlet extractor for 17 h. After evaporation of the solvent, 20 g of CH_2Cl_2 crude extract was subjected to column chromatography over silica gel (gradient solvent system; $CHCl_3$ and MeOH) to give 28 fractions. Fractions 23-27, then were repeated CC on ODS silica gel (20% MeOH, 80% H_2O, 0.1% acetic acid) afforded kopsininic acid (**3**, 30 mg). Fractions 12-18 (10 g) also was purified by CC (NH silica, ODS silica and normal silica gel) yielded a small amount of singaporentine A (**1**, 2.6 mg). PTLC on CH_2Cl_2 crude extract (40 mg) afforded kopsifoline A (**2**, 11.6 mg).

Singaporentine A (1): light yellowish oil, with $[\alpha]_D^{26}$ -23 (*c* 0.3, MeOH); UV (MeOH) λ_{max} 232 (4.06) , 253 (3.63), and 310 (3.57) nm; IR (liquid film) λ_{max} 2970, 1738 and 1720 (C=O), and 1217 cm^{-1}; HRESIMS *m/z* 379.1659 ($[M + H]^+$; calcd. for $C_{22}H_{23}N_2O_4$, 379.1658). ^{1}H-NMR and ^{13}C-NMR see Table 1.

Cytotoxic assays. P-388 murine leukemia cells were maintained in RPMI-1640 medium supplemented with 5% fetal calf serum and kanamycin (100 μg/mL). The cells (3×10^3 cells/well) were cultured in Corning disposable 96-well plates containing 100 μL of growth medium per well and were incubated at 37°C in a humidified atmosphere of 5% CO_2. Various drug concentrations (10 μL) were added to the cultures at day one after the transplantation. At day three, 20 μL MTT solution (5 mg/mL) per well was added to each cultured medium. After a further 4 h of incubation, 100 mL of 10% SDS-0.01N HCl solution was added to each well and the formazan crystals in each well were dissolved by stirring with a pipette. The optical density measurements were made using a micropipette reader (Tohso MPR-A4i) with a two wavelength system (550 and 700 nm). In all experiments, three replicate wells were used to determine each point.

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