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New Indole Alkaloids from *Kopsia Singapurensis* (RIDL.)

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Abstract: Study on chemical constituents from *Kopsia singapurensis* (Apocynaceae) yielded four new aspidofractinines: Singaporentine A **(1)**, *N*(1)-formylkopsininic acid (**2**), *N*(1)-formylkopsininic acid-*N*(4)-oxide (**3**), 15-hydroxykopsamine (**4**); a new aspidospermatan: 14α-hydroxy-*N*(4)-methylcondylocarpine (**5**) and a new akuamiline: singaporentinidine (**6**) together with 18 known indole alkaloids from different parts of the plant. The structures of these compounds were elucidated by combination of various spectroscopic methods.

Keywords: Indole, aspidofractinines, aspidospermatan, akuamiline, Apocynaceae.

INTRODUCTION

The genus *Kopsia* (Apocynaceae) comprises of 30 species which is native to China, India and Southeast Asia [1-7]. In Malay Peninsula, *Kopsia singapurensis* Ridl. is one of the 18 *Kopsia* species that are distributed from Negeri Sembilan southward to Singapore and common in lowland swampy forest [8, 9]. This species is known to produce a large number of biologically active indole alkaloids possessing interesting skeletons [1-7]. 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 leaves, roots and barks of *Kopsia singapurensis* Ridl. have afforded four new aspidofractinines: Singaporentine A (1), *N*(1)-formylkopsininic acid (2), *N*(1)-formylkopsininic acid-*N*(4)-oxide (3), 15-hydroxykopsamine (4); a new aspidospermatan: 14α-hydroxy-*N*(4)-methylcondylocarpine (5) and a new akuamiline: singaporentinidine (6) together with 18 known indole alkaloids; from bark: venalstonine, venacarpine A, rhazinilam, pleicarpamine, 16-epideacetylakuammiline, 15-hydroxykopsinine, 16-hydroxymethylpleiocarpamine, lonicerine, kopsinine-*N*-oxide and 16-epideacetylakuammiline-*N*-oxide; from leaves: kopsininic acid and kopsifoline A; from roots: kopsamine *N*(4)-oxide, 16-epiakuammiline, *N*-methylpleiocarpamine, aspidodasycarpine, kopsamine, kopsinine and kopsininic acid. Their structures were elucidated by combination of various spectroscopic methods such as 1D and 2D NMR, IR, UV and MS. We will only describe the isolation and structural elucidation of these new compounds in the present paper.

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EXPERIMENTAL

General Experimental Procedures

Spectra were recorded on the following instruments. Optical rotations at 25 °C were taken on Jasco DIP-1000 Digital polarimeter. UV spectra were recorded on a Shimadzu UVmini-1240 spectrophotometer and IR spectra on a Perkin Elmer 1600 spectrophotometer. UV, IR and optical rotation were recorded in methanol. CD spectra were recorded on a JASCO J-820 polarimeter. Mass spectra were obtained using LC-EIMS, Waters Micromass ZQ and a LTQ Orbitrap XL (Thermo Scientific) spectrometer. NMR spectra were recorded on a Bruker Avance 600 spectrometer and chemical shifts were reported using residual CD_3OD (δ_H 3.31 and δ_C 49.0) as internal standards. HPLC was performed on a C18 MG-II (ϕ 10 mm l.D x 250 mm).

Plant Material

The barks and leave of *Kopsia singapurensis* were collected in Kluang, Johor, Malaysia in 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. The roots of *Kopsia singapurensis* were collected in Kluang, Johor, Malaysia in

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2010. Identification was made by Mr. Teo Leong Eng, University of Malaya. Voucher specimens (KL 5724) were deposited at Herbarium of the Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia.

Extraction and Isolation

The dried bark (3 kg) of *Kopsia singapurensis* were first defatted in hexane by Soxhlet extractor for 17 hours. Then the extract was dried on the rotary evaporator. The plant material was dried and wetted with 10 % ammonia solution and left for overnight. They were then re-extracted successively with CH_2Cl_2. After removal of the solvents, 50.0 g CH_2Cl_2 crude extracts were obtained for the bark. The crude extract of the bark were then dissolved in hexane for three days to give crude which is dissolved in hexane (9.0 g). After evaporation of the solvent, 9.0 g of the crude was subjected to column chromatography over silica gel (gradient solvent system; $CHCl_3$ and MeOH) yielded 10 known compounds: venalstonine, venacarpine A, rhazinilam, pleicarpamine, 16-epideacetylakuammiline, 15-hydroxykopsinine, 16-hydroxymethylpleiocarpamine, lonicerine, kopsinine-*N*-oxide and 16-epideacetylakuammiline-*N*-oxide.

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 hours. 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 and ODC silica gel (20% MeOH, 80% H_2O, 0.1% acetic acid) afforded kopsininic acid (30.0 mg). Fractions 12-18 (10 g) was purified by CC (NH silica, ODS silica and normal silica gel) yielded a small amount of singaporentine A (**1**, 2.6 mg). PTLC and CH_2Cl_2 crude extract (40.0 mg) afforded kopsifoline A (11.6 mg).

The dried roots (1 kg) of *Kopsia singapurensis* were ground and extracted exhaustively with MeOH to give 35.0 g of MeOH crude extract. The MeOH crude extract (20.0 g) were further extracted with EtOAc / 3% tartaric acid (pH 2), $CHCl_3$/saturated Na_2CO_3 (pH 10) to yielded EtOAc crude extract (15.0 g) and alkaloid crude extract (4.0 g) respectively. The alkaloidal fraction (2.59 g) was subjected to a Sephadex LH-20 column with solvent system $CHCl_3$/MeOH (1:1) to give 20 fractions. Each series of fractions was then treated separately by extensive column chromatography. Fractions I and J (190.0 mg) was further purified by an ODS column (MeOH/H_2O + 0.1% formic acid, 2:8 % 1:0) afforded *N*(1)-formylkopsininic acid (**2**, 18.8 mg) and *N*(1)-formylkopsininic acid- *N*(4)-oxide (**3**, 6.6 mg) together with kopsamine *N*(4)-oxide (11.5 mg). Further purification on fractions eluted by the ODS column with an ODS HPLC (MeCN/H_2O + 0.1% formic acid, 2:8, flow rate 2mL/min; UV detection at 220 nm, Rt 15.0 min, 17.0 min and 21.0 min) to give 14α-hydroxy-*N*(4)-methylcondylocarpine (**5**, 5.3 mg) together with 16-epiakuammiline (2.4 mg), *N*-methylpleiocarpamine (7.6 mg) and aspidodasycarpine (81.1 mg). The work-up procedure on fractions M and N (680.0 mg) with normal silica and followed by ODS column with an ODS HPLC (MeCN/H_2O + 0.1% formic acid, 2:8, flow rate 2mL/min; UV detection at 220 nm, Rt 18.0 min and 23.0 min) to give 15-hydroxykopsamine (**4**, 2.4 mg) and singaporentinidine (**6**, 3.5 mg) together with kopsamine (5.2 mg), kopsinine (14.8 mg) and kopsininic acid (2.0 mg).

Singaporentine A (1): light yellowish oil, with $|\alpha|_D^{28}$ –23 (*c* 0.3, MeOH); UV (MeOH) λ_{max} 232, 253 and 310 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). ^1H and ^{13}C NMR see Table **1** and Table **2**.

***N*(1)-Formylkopsininic acid (2)**: yellowish amorphous, with $|\alpha|_D^{28}$ –304 (*c* 0.25, MeOH); UV (MeOH) λ_{max} 200, 240, and 290 nm; IR (liquid film) λ_{max} 3400 (OH), 1730 and 1710 (C=O), and 1616 cm^{-1}; HRESIMS *m/z* 353.18391 ($[M+H]^+$; calcd. for $C_{21}H_{25}N_2O_3$, 353.18652). ^1H and ^{13}C NMR see Table **1** and Table **2**.

***N*(1)-Formylkopsininic acid-*N*(4)-oxide (3)**: light yellowish amorphous, with $|\alpha|_D^{28}$ –93 (*c* 0.25, MeOH); UV (MeOH) λ_{max} 200, 240 and 290 nm; IR (liquid film) λ_{max} 3450 (OH), 1720 (C=O) and 1614 cm^{-1}; HRESIMS *m/z* 369.17966 ($[M+H]^+$; calcd. for $C_{21}H_{25}N_2O_4$, 369.18143). ^1H and ^{13}C NMR see Table **1** and Table **2**.

15-Hydroxykopsamine (4): yellowish amorphous, with [illegible] –19 (*c* 0.12, MeOH); UV (MeOH) λ_{max} 203, 226 and 290 nm; IR (liquid film) λ_{max} 3450 (OH) and 1710 (C=O) cm^{-1}; HRESIMS *m/z* 473.1934 ($[M+H]^+$; calcd. for $C_{24}H_{29}N_2O_8$, 473.1924). ^1H and ^{13}C NMR see Table **1** and Table **2**.

14α-Hydroxy-N(4)-methylcondylocarpine (5): light yellowish amorphous, with $|\alpha|_D^{28}$ +386 (*c* 0.25, MeOH); UV (MeOH) λ_{max} 200, 224, 290 and 327 nm; IR (liquid film) λ_{max} 3460 (NH/OH) and 1700 (C=O) cm^{-1}; HRESIMS *m/z* 353.18396 ($[M+H]^+$; calcd. for $C_{21}H_{25}N_2O_3$, 353.18652). ^1H and ^{13}C NMR see Table **1** and Table **2**.

Singaporentinidine (6): light yellowish amorphous, with $|\alpha|_D^{28}$ –2 (*c* 0. 175 MeOH); UV (MeOH) λ_{max} 200, 220, 280 and 327 nm; IR (liquid film) λ_{max} 3440 (NH/OH) and 1730 (C=O) cm^{-1}; HRESIMS *m/z* 309.1577 ($[M]^+$; calcd. for $C_{19}H_{21}N_2O_2$, 309.1598). ^1H and ^{13}C NMR see Table **1** and Table **2**.

RESULTS AND DISCUSSION

Singaporentine A **(1)** was isolated from dichloromethane extract of the leaves of *K. singapurensis* as a light yellowish oil, with $|\alpha|_D^{28}$ -23^o (*c* = 0.3, MeOH). The UV spectrum showed the maximum absorptions at 232, 253 and 310 nm indicating the presence of an indolenine chromophore [13,14]. The IR spectrum showed the absorption bands at 1738 and 1720 cm^{-1} which were assigned to the present of a carbonyl groups for methyl ester and lactam [15], respectively.

Table 1. ^{1}H NMR [600 MHz, δ_H (*J* Hz)] of 1 - 6 in $CDCl_3$

Position	δH, *J* Hz					
	1	**2**	**3**	**4**	**5**	**6**
NH					8.52 (br, s)	
2						
3	3.62 (*d*, 18.0)	3.20 (m)	3.93 (d, 11.0)	3.01 (m)	3.47 (m)	4.97 (br, s)
	4.60 (*d*, 18.0)	3.27 (m)	4.17 (d, 11.0)	3.10 (m)	3.85 (m)	
5		3.39 (t, 10.0)	3.80 (d, 10.0)	3.00 (m)	3.70 (m)	3.59 (m)
		3.52 (t, 10.0)	3.96 (d, 10.0)	3.05 (m)	3.74 (m)	4.86 (m)
6	2.56 (*d*, 18.0)	1.74 (m)	1.99 (m)	1.66 (m)	2.23 (m)	3.09 (m)
	3.26 (*d*, 18.0)	2.80 (m)	2.98 (m)	2.12 (m)	3.13 (m)	
7						
8						
9	6.93 (*d*, 8.0)	7.50 (d, 8.0)	7.68 (d, 8.0)	6.77 (d, 8.0)	7.59 (d, 7.0)	7.46 (d, 8.0)
10	7.22 (*t*, 8.0)	6.71 (t, 8.0)	6.77 (t, 8.0)	6.52 (d, 8.0)	6.97 (t, 7.0)	7.08 (t, 8.0)
11	6.9 (d, 8.0)	6.96 (t, 8.0)	7.00 (t, 8.0)		7.21 (t, 7.0)	7.17 (t, 8.0)
12		6.68 (d, 8.0)	6.66 (d, 8.0)		6.98 (d, 7.0)	7.34 (d, 8.0)
13						
14	5.74 (d, 10.0)	1.64 (m)	1.88 (m)	1.53 (m)	4.18 (br, s)	2.34 (d, 7.0)
		1.89 (m)	1.93 (m)	1.79 (m)		
15	5.63 (d, 10.0)	1.37 (m)	1.42 (m)	3.45 (dd, 4.0, 12.0)	3.61 (br, s)	3.80 (s)
		1.62 (m)	1.85 (m)			
16		2.85 (m)	2.93 (m)			4.08 (s)
17	1.81 (d, 12.4)	1.60 (m)	1.53 (m)	1.89 (d, 15.0)		
	2.24 (d, 13.2)	2.56 (m)	2.63 (m)	2.58 (dd, 2, 15.0)		
18	2.37 (m)	1.39 (m)	1.36 (m)	1.51 (m)	1.75 (d, 7.0)	1.73 (d, 7.0)
	2.42 (m)	1.84 (m)	1.65 (m)	2.38 (t, 11.0)		
19	1.21 (m)	1.35 (m)	1.51 (m)	1.12 (t, 11.0)	5.95 (q, 7.0)	5.49 (q, 7.0)
	1.74 (m)	1.57 (m)	1.59 (m)	2.17 (m)		
20						
21	3.49	3.60 (s)	4.01 (s)	2.89 (s)	5.37 (br, s)	3.97 (d, 14.0)
						4.88 (m)
22	3.73 (s)				3.81 (s)	
23		8.40 (s)	8.43 (s)	3.76 (s)	3.51 (s)	
24	3.97 (s)					
25				3.89 (s)		
26				5.90 (s)		
16-OH				6.92 (br, s)		

Table 2. ^{13}C NMR [150 MHz, δ_C] of 1 - 6 in $CDCl_3$

Position	δC					
	1	2	3	4	5	6
NH						
2	183.3	65.2	65.1	74.8	167.8	129.8
3	40.0	46.6	63.2	45.9	61.5	70.1
5	171.1	49.9	63.8	50.3	64.5	51.7
6	39.1	33.6	32.4	37.3	41.5	18.5
7	55.3	58.2	58.8	57.6	58.3	104.9
8	146.7	137.4	137.4	123.3	133.3	125.9
9	112.8	122.7	124.3	114.5	121.0	118.8
10	128.5	122.3	121.5	103.9	122.8	120.4
11	111.5	127.9	128.1	148.4	130.3	123.3
12	151.4	113.1	112.7	134.2	112.0	112.1
13	142.3	147.1	148.3	135.7	145.9	138.0
14	123.9	15.1	19.3	26.4	67.1	34.8
15	132.4	33.3	31.9	76.2	44.4	42.6
16	56.6	42.1	42.8	74.6	101.0	72.3
17	41.7	32.8	31.8	34.5	168.0	168.0
18	39.6	31.6	34.3	23.5	13.3	14.4
19	33.0	33.8	33.0	28.2	130.8	119.3
20	45.0	30.9	34.0	38.9	125.9	132.7
21	64.8	66.8	83.1	67.8	72.1	66.2
22	52.7	177.0	177.1	172.9	52.0	
23	171.2	167.6	167.3	52.5	51.7	
24	55.5			156.1		
25				53.3		
26				100.3		
16-OH						

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). ^1H and ^{13}C NMR data (Table **1** and Table **2**) 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 ^1H-^1H 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 ^1H 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) [15]. 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

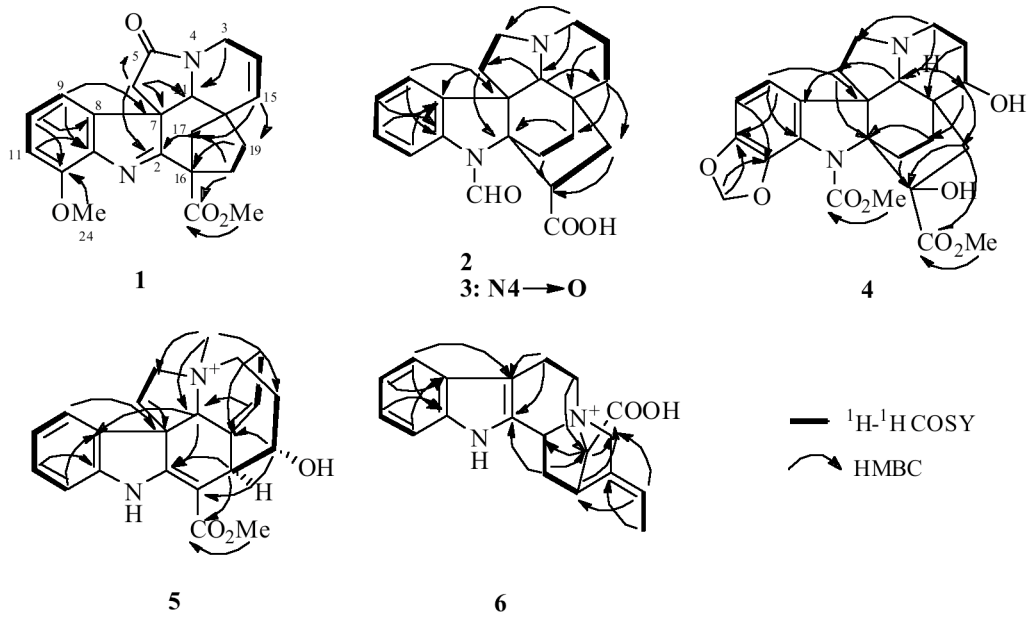


Fig. (1). Selected 2D NMR correlation for 1, 2, 4, 5 and 6.

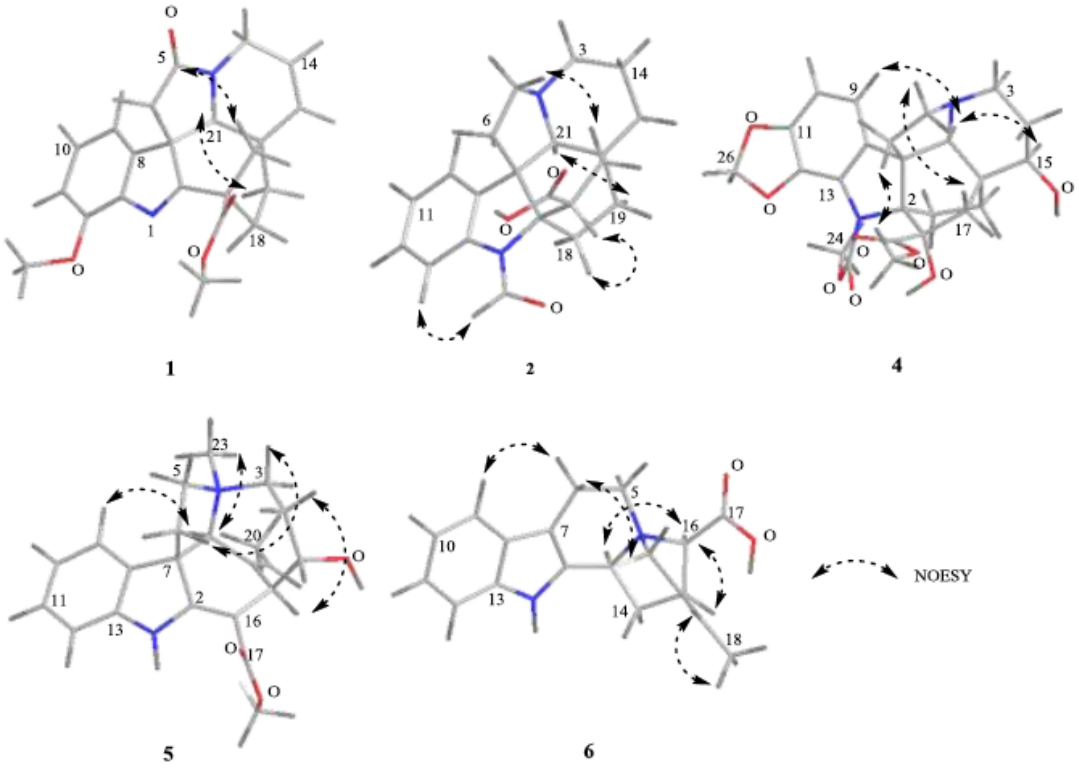


Fig. (2). Selected NOSEY correlations for 1, 2, 4, 5 and 6.

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 (OCH_3-23) 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** (Fig. **1**). The relative stereochemistry of **1** was established by NOESY correlations as shown in the computer-generated 3D drawing (Fig. **2**). NOESY correlations of H-6/H-17β and H-19α/H-21 indicated that singaporentine A possessed the same stereochemistry with kopsifoline E [16], previously isolated from the leaf of *K. fruticosa*. Therefore the relative stereochemistry of **1** is as depicted in Fig. (**2**).

N(1)-Formylkopsininic acid **(2)** was isolated from the alkaloid crude extract of the roots of K. singapurensis as yellowish amorphous with $|\alpha|\frac{28}{D}$ −304 (c 0.25, MeOH). It

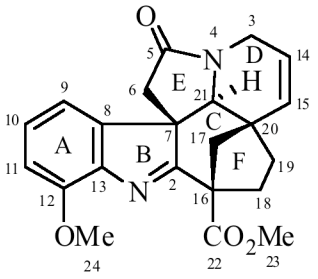
was revealed to have the molecular formula $C_{21}H_{24}N_2O_3$, by HRESITOFMS [m/z 353.18391 $(M+H)^+$, Δ −2.61 mmu]. The UV spectrum showed absorptions at 240 and 290 nm which showed characteristic of an indoline chromophore [17,18]. The IR spectrum indicated absorptions for a hydroxyl group (3400 cm^{-1}), two carbonyl groups (1730 cm^{-1} and 1710 cm^{-1}) and aromatic ring (1616 cm^{-1}). The ^1H and ^{13}C NMR spectra (Table **1** & Table **2**) of **2** resembled those of kopsininic acid, which was also isolated from the leaves extract of the same plant [17], with an additional signal indicative of a formamide group (δ_H 8.40 and δ_C 167.6). The structure of **1** as the N(1)-formyl derivative of kopsininic acid was confirmed by analysis of the 2D NMR data (Fig. **1**) as follows. The ^1H-^1H COSY correlations revealed the presence of -CHCHCHCH- (C-9~C-12), -CH_2CH_2-(C-5, C-6), -$CH_2CH_2CH_2$- (C-3, C-14, C-15), -CH_2CH_2- (C-18, C-19), and -$CHCH_2$- (C-16, C-17) fragments. HMBC correlations of H-6 to C-2 and C-8, H-9 to C-7 and C-13, H-12 and H-10 to C-8 confirmed the presence of the indoline ring. The connectivity of C-3, C-5 and C-21 through a nitrogen atom was deduced from the HMBC cross-peaks of H2-3 to C-5 and C-21. The HMBC correlations of H2-18 to C-16, H2-19 to C-2 and H-21 to C-6 suggested the connectivity of C-16 and C-18 to C-2 and C-21 to C-7. The HMBC correlations from H2-14 to C-20, H-15 to C-17 and C-19, and H-21 to C-15 indicated the connectivity of C-15, C-17, C-19 and C-21 through C-20. Finally, the presence of a formamide (δ_H 8.40; δ_C 167.6) attached to the nitrogen atom (N-1) was indicated by a NOESY correlation of H-12/CHO (Fig. **2**), and the presence of a hydroxylcarbonyl connected to C-16 was deduced from the HMBC correlations of H2-17 to C-22. The relative configuration of 2 was deduced by NOESY correlations as shown in the computer-generated 3D drawing (Fig. **2**). The NOESY correlations of H-5a/H-17b and H-19a/H-21 established the relative configuration of C-2, C-7, C-20 and C-21. The orientation of H-16 was deduced to be α from the NOESY correlations of H-16/H-18b. Therefore, the relative configuration of **2** was assigned to be a depicted in Fig. (**2**).

N(1)-Formylkopsininic acid-*N*(4)-oxide **(3)** was isolated as yellowish amorphous, with $|\alpha|\frac{28}{D}$ −93 (*c* 0.25, MeOH)}, form the alkaloid crude extract of *K. singapurensis*. It showed a pseudo-molecular ion peak at *m/z* 369.17966 ($[M+H]^+$, Δ −1.77 mmu), which is consistent to the molecular formula $C_{21}H_{24}N_2O_4$, differing from **2** by addition of one oxygen atom. The similar IR and UV spectra to **2** were observed for **3**. Comparison of the ^1H and ^{13}C NMR data of **3** with **2** (Table **1** & Table **2**) suggested that **3** is closely related to **2** except for the characteristic downfield chemical shifts involving protons and carbons at position 3 (δ_H 3.93 and 4.17, δ_C 63.2), 5 (δ_H 3.80 and 3.96, δ_C 63.8) and 21 (δ_H 4.01, δ_C 83.1), indicating the presence of *N*(4)-oxide.

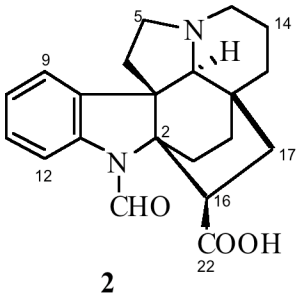
15-Hydroxykopsamine **(4)** was isolated as yellowish amorphous with $|\alpha|\frac{28}{D}$ −19 (*c* 0.12, MeOH)} showed a molecular formula $C_{24}H_{28}N_2O_8$, which was determined by HRESITOFMS [*m/z* 473.1934 $(M+H)^+$, Δ +1.0 mmu]. The IR absorption band at 3450 cm^{-1} was characteristic of amino or hydroxy group and the band at 1710 cm^{-1} indicated the presence of a carbonyl group. The UV spectrum showed the maximum absorption at 203, 226 and 290 nm which were characteristic of a indoline chromophore [17, 18]. The NMR data for **4** resembled those of kopsamine which was isolated from the leaves extract of *K. pauciflora* Hook f [16]. The significant difference between both was the presence of an oxymethine signal (δ_H 3.80, s; δ_C 76.2) in place of the CH_2-15 signal of kopsamine. Thus, **4** was assumed to be a 15 hydroxy derivative of kopsamine, and this assumption was further confirmed by the HMBC correlations of H-15 with C-3 and C-21 (Fig. **1**). The relative configuration of **4** was established by NOESY correlations (Fig. **2**) to be similar to kopsamine, with the NOESY correlation of H-15/H-21 indicated that H-15 took an α-orientation. Finally, C-15 was determined to have the *R*-configuration by employing the advanced Mosher's method.

14α-Hydroxy-*N*(4)-methylcondylocarpine **(5)** was isolated as light yellowish amorphous with $|\alpha|\frac{28}{D}$ +386 (*c* 0.25, MeOH)} which showed molecular ion peak at *m/z* 353.18396 ([M]+, Δ −2.56 mmu). It was consistent to the molecular formula $C_{21}H_{25}N_2O_3$. Its UV absorption maxima at 224, 290, and 327 nm suggested the presence of an anilinoacrylate chromophore [5,19]. The IR spectrum showed absorption band at 3460 cm^{-1} and 1700 cm^{-1} indicating the presence of an amine and/or a hydroxyl and an ester carbonyl groups, respectively. The ^1H and ^{13}C NMR data (Table **1** and Table **2**) were reminiscent of those of 14α-hydroxycondylocarpine [20] except for the additional methyl signal (δ_H 3.81, δ_C 51.7) and the downfield chemical shifts of protons and carbons at position 3 (δ_H 3.47 and 3.85, δ_C 61.5), 5 (δ_H 3.70 and 3.74, δ_C 64.5) and 21 (δ_H 5.37, δ_C 72.1), suggesting the presence of an *N*(4)-methyl. The position of the additional methyl was verified by HMBC correlations from H3-23 to C-3, C-5, and C-21 (Fig. **1**) and the relative configuration of 4 was deduced by NOESY correlations to be the same as 14α-hydroxycondylocarpine (Fig. **2**). Thus, compound 5 was concluded to be 14α-hydroxy-*N*(4)-methylcondylocarpine.

Singaporentinidine **(6)** with $|\alpha|\frac{28}{D}$ −2 (*c* 0.175, MeOH)} was isolated as light yellowish amorphous. It showed a molecular formula $C_{19}H_{21}N_2O_2$, which was determined by HRESITOFMS [m/z 309.1577 (M)+, Δ −2.1 mmu]. The IR absorption at 3440 cm^{-1} was indicating the presence of amino or hydroxyl group and the band at 1730 cm^{-1} indicated the presence of a carbonyl group. The UV spectrum revealed the maximum absorption at 200, 220, 280 and 327 nm which were characteristic of an indole chromophore [17, 18]. Analysis of the 1D and 2D NMR data of 6 (Fig. **1**) revealed a planar structure which is related to excelsinidine [21] isolated from *Aspidosperma excelsum*, and the difference was the presence of a proton at C-16 in 5

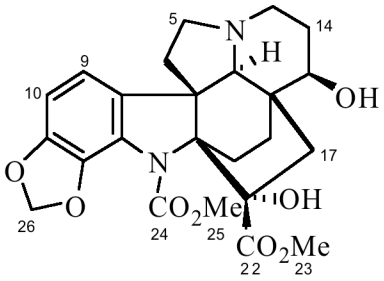


1

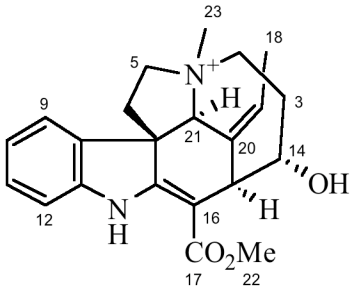


2

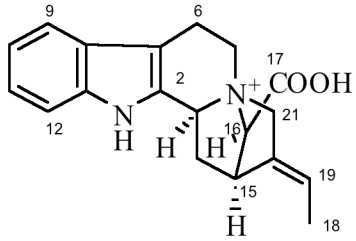
3: N4→O



4



5



6

instead of a hydroxymethyl in excelsinidine. Analysis of the NOESY data (Fig. **2**) established the relative configuration of 6. The E configuration of C-19 double bond was deduced from the NOESY correlations of H-15/H3-18 and H-19/H-21a. The α-orientation of C-3 was suggested by NOESY cross-peaks between H-3/H-21b and the orientation of H-16 was deduced from the NOESY correlation of H-6/H-16. Thus, the relative configuration of 5 was assigned to be as depicted in Fig. (**2**).

Biogenetically, the skeleton of 1-6 can be derived from the corynantheine skeleton. C-16 ~ N-4 cyclization of a corynantheine skeleton would yield an akuammiline skeleton as in 6. Rearrangements of a corynantheine skeleton may yield a stemmadenine skeleton, of which the aspidofractinine (1-4) and aspidospermatan (5) skeleton can be derived.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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