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FLAVISIAMINES A – D, NEW INDOLE ALKALOIDS FROM *KOPSIA FLAVIDA*

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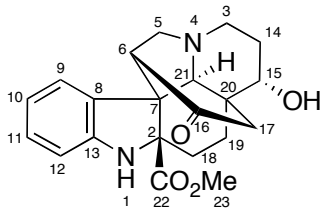
Abstract – Four new methyl chanofruticosinate-type alkaloids, flavisiamines A (**1**) ~ D (**4**) have been isolated from *Kopsia flavida* (Apocynaceae) and their structures were elucidated by NMR spectral analysis using 2D techniques.

INTRODUCTION

Indole alkaloids with unique skeletons often show some interesting biological activities.[1] In our research for structurally unique and biologically interesting indole alkaloids, we previously isolated quaternary indole alkaloids, subincanadines from barks of *Aspidosperma subincanum* (Apocynaceae).[2] On the other hand, the genus *Kopsia* (Apocynaceae), which is widely distributed throughout tropical Asia, is noted for producing variety of indole alkaloids with useful biological activities.[3,4] Recent investigation of extracts from the leaves of *K. flavida* resulted in the isolation of new indole alkaloids, flavisiamines A (**1**) ~ D (**4**) together with five known indole alkaloids. In this paper, we report the isolation and structure elucidation of flavisiamines A (**1**) ~ D (**4**).

The leaves of *K. flavida* were extracted with MeOH, and the MeOH extract was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, which were adjusted to pH 10 with saturated Na_2CO_3, were extracted with $CHCl_3$. $CHCl_3$-soluble materials were subjected to a silica gel column ($CHCl_3$/MeOH 1:0 → 0:1). $CHCl_3$/MeOH (10:1) eluted fractions were purified by C_{18} HPLC to afford flavisiamines A (**1**, 0.010%), B (**2**, 0.007%), C (**3**, 0.008%), and D (**4**, 0.011%), together with known compounds, *N*1-decarbomethoxychanofruticosinate,[5] methyl 11,12-methylenedioxy-*N*1-decarbomethoxychanofruticosinate,[5] methyl 11,12-methylenedioxy-*N*1-decarbomethoxy-$\Delta^{14,15}$-chanofruticosinate,[5] methyl 12-methoxy-decarbomethoxychanofruticosinate,[4a] oxychanofruticosinate.[4a]



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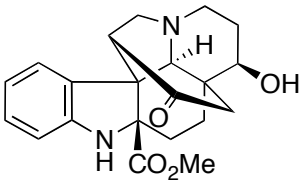

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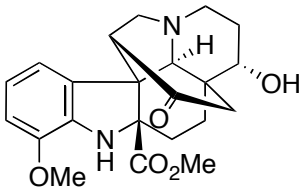

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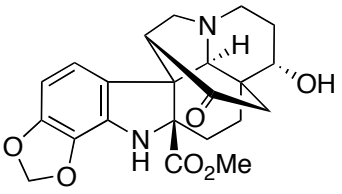

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RESULTS AND DISCUSSION

Flavisiamine A (**1**) showed the pseudomolecular ion peak at *m/z* 369 $(M+H)^+$ in ESIMS, and the molecular formula, $C_{21}H_{24}N_2O_4$, was established by HRESIMS [*m/z* 369.1818, $(M+H)^+$ Δ +0.4 mDa]. IR spectrum suggested the presence of OH (3346 cm^{-1}) and carbonyl (1732 cm^{-1}) groups. The ^{13}C NMR spectrum (Table 1) of **1** disclosed twenty-one carbon signals due to one carbonyl (δ_C 205.6), one ester carbonyl (δ_C 174.3), two *sp*^{2} quaternary carbons (δ_C 150.2 and 129.6), three *sp*^{3} quaternary carbon (δ_C 76.0, 60.4, and 39.6), four *sp*^{2} methines (δ_C 131.2, 124.0, 120.8, and 111.9), three *sp*^{3} methines (δ_C 67.7, 66.1, and 54.1), six *sp*^{3} methylenes (δ_C 53.6, 45.3, 43.8, 32.0, 27.4, and 24.0), and one methyl (δ_C 52.8) attached to an oxygen atom. Proton and carbon signals for **1** were assigned by detailed analysis of the HSQC spectrum. The ^1H-^1H COSY spectrum revealed connectivities of C-3 - C-15, C-5 to C-6, C-9 - C-12, and C-18 to C-19 (Figure 1). HMBC correlations of H-9 (δ_H 7.22) to C-13 (δ_C 150.2) and C-7 (δ_C 60.4), H-12 (δ_H 6.84) to C-8 (δ_C 129.6), H-21 (δ_H 3.77) to C-2 (δ_C 76.0), C-7, C-8, and C-19 (δ_C 32.0), and H_2-18 (δ_H 2.16 and 1.86) to C-7 and C-20 (δ_C 39.6) revealed the presence of a hexahydrocarbazole ring (C-2, C-18 - C-21, C-7 - C-13, and *N*-1). Cross peaks of H-21 to C-5 (δ_C 53.6) and C-6 (δ_C 54.1), and H-6 (δ_H 3.60) to C-7 in the HMBC spectrum implied the presence of a pyrrolidine ring (C-5 - C-7, C-21, and *N*-4). The presence of a piperidine ring (C-3, C-14, C-15, C-20, C-21, and *N*-4) with a hydroxy group at C-15 was deduced from the HMBC correlations of H-21 to C-3 (δ_C 45.3) and C-15 (δ_C 67.7) and H_2-14 (δ_H 2.64 and 1.86) to C-20. HMBC correlations of H-6 (δ_H 3.60) to C-16 (δ_C 205.6), and H-17a (δ_H 2.93) to C-16 and C-20 revealed an azepane ring (C-5, C-6, C-16, C-17, C-20, C-21, and *N*-4) with a ketone at C-16. HMBC correlations of H-18 and H_3-23 (δ_H 3.56) to C-22 (δ_C 174.3) were implied methoxy carbonyl group connected at C-2. Thus, the structure of flavisiamine A was elucidated to be **1**. These data suggested that flavisiamine A possessed methyl chanofruticosinate skeleton[5] with a hydroxyl at C-15, a methoxy carbonyl group at C-2, and a ketone at C-16. The relative stereochemistry of **1** was elucidated by ROESY correlations as shown in Figure 2. ROESY correlations of H-15 to H-17a indicated both a hydroxyl group at C-15 and H-21 were α-orientation. Thus, the relative stereochemistry of **1** was assigned as shown in Figure 2.

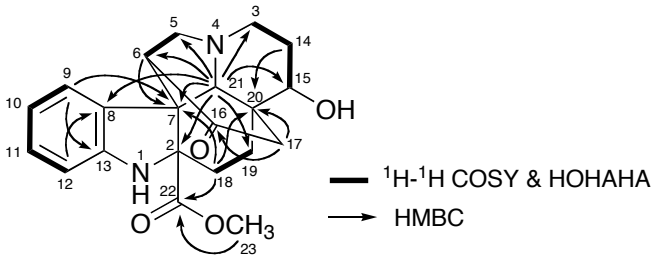

Figure 1. Selected 2D NMR correlations for flavisiamine A (**1**)

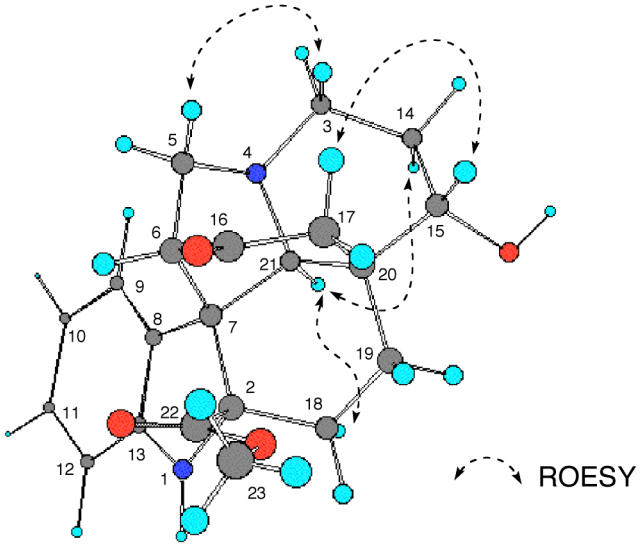

Figure 2. Selected ROESY correlations for flavisiamine A (**1**)

Flavisiamine B (**2**) showed the pseudomolecular ion peak at *m/z* 369 $(M+H)^+$ in ESIMS, and the molecular formula, $C_{21}H_{24}N_2O_4$, was established by HRESIMS [*m/z* 369.1812, $(M+H)^+$ Δ -0.2 mDa], which was the same as that of **1**. ^{1}H and ^{13}C NMR data (Tables 1 and 2) of **2** were quite similar to those of **1**. Detailed analysis of 2D NMR data (^{1}H-^{1}H COSY, HSQC, and HMBC) suggested that gross structure of **2** was the same as that of **1**. Hydroxyl group at C-15 was deduced to be β-orientation by a ROESY correlation between H-15 and H-19α. Thus, flavisiamine B (**2**) was assigned as an epimer at C-15 of flavisiamine A.

Flavisiamines C (**3**) and D (**4**) showed the pseudomolecular ion peak at *m/z* 399 $(M+H)^+$ and 413 $(M+H)^+$, respectively in ESIMS, and the molecular formulae were established to be $C_{22}H_{26}N_2O_5$ and $C_{22}H_{24}N_2O_6$ by HRESIMS [**3**: *m/z* 399.1917 $(M+H)^+$ Δ -0.3 mDa; **4**: *m/z* 413.1690 $(M+H)^+$ Δ -2.3 mDa], respectively. Based on the ^{1}H NMR and ^{13}C NMR spectra (Tables 1 and 2), NMR signal patterns of **3** and **4** were very similar to those of **1** except for methoxy and methylenedioxy groups, respectively. HMBC cross peaks

indicated the presence of a methoxy group attached to C-12 in **3** and a methylenedioxy group to C-11 and C-12 in **4**. The proton coupling patterns support the structural assignment of **3** and **4**, and the same relative stereochemistry as in **1** was supported by ROESY correlations. Thus, flavisiamines C and D were assigned to be **3** and **4**, respectively as shown in Figure.

Table 1. ^{1}H NMR Data [δ_H (J, Hz)] of Flavisiamines A (**1**) ~ D (**4**) in CD_3OD.

Position	**1**	**2**	**3**	**4**
3a	3.65 (dd, 12.6, 5.6)	3.48 (m)	3.64 (m)	3.67 (m)
3b	3.37 (dd, 12.6, 5.6)	3.47 (m)	3.31 (m)	3.35 (m)
5a	4.49 (dd, 14.8, 6.9)	4.40 (dd, 13.9, 7.2)	4.41 (dd, 12.5, 5.6)	4.44 (dd, 13.8, 1.7)
5b	3.83 (d, 14.8)	3.79 (d, 13.9)	3.77 (m)	3.80 (m)
6	3.60 (d, 6.9)	3.57 (m)	3.56 (m)	3.50 (m)
9	7.22 (d, 7.3)	7.20 (d, 7.6)	6.88 (d, 7.6)	6.75 (d, 7.9)
10	6.82 (t, 7.3)	6.81 (d, 7.6)	6.85 (t, 7.6)	6.36 (d, 7.9)
11	7.19 (t, 7.3)	7.17 (d, 7.6)	6.92 (d, 7.6)	
12	6.84 (d, 7.3)	6.79 (d, 7.6)		
14a	2.64 (d, 15.9, 5.6, 2.9)	2.28 (m)	2.63 (dt, 14.6, 4.5)	2.63 (dt, 14.5, 5.7)
14b	1.86 (m)	1.97 (m)	1.84 (dt, 14.6, 5.0)	1.86 (m)
15	3.66 (brs)	3.70 (dd, 10.8, 4.4)	3.65 (m)	3.66 (m)
17a	2.93 (d, 19.3)	3.11 (d, 19.0)	2.91 (d, 19.3)	2.92 (d, 19.3)
17b	2.38 (d, 19.3)	2.28 (d, 19.0)	2.38 (d, 19.3)	2.38 (d, 19.3)
18a	2.16 (ddd, 15.5, 5.7, 1.8)	2.15 (m)	2.20 (dd, 15.5, 2.9)	2.18 (ddd, 14.1, 4.2, 1.5)
18b	1.86 (m)	1.81 (m)	1.87 (dd, 15.5, 5.3)	1.90 (m)
19a	2.32 (dt, 13.8, 5.7)	2.15 (m)	2.33 (dd, 13.5, 4.7)	2.32 (dt, 13.5, 5.0)
19b	1.53 (ddd, 13.8, 3.0, 1.4)	1.81 (m)	1.52 (ddd, 13.5, 3.2, 2.1)	1.53 (dd, 13.5, 4.4)
21	3.77 (brs)	3.57 (m)	3.74 (m)	3.77 (brs)
23	3.56 (s)	3.56 (s)	3.56 (s)	3.59 (s)
OMe			3.86 (s)	
OCH_2O				5.97 (brs), 5.90 (brs)

EXPERIMENTAL

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were obtained on a Varian INOVA 600 spectrometer using TMS as an internal standard. HSQC experiments were optimized for $^1J_{CH}$ = 140 Hz and HMBC experiments for $^nJ_{CH}$ = 8 Hz. Positive-mode ESI mass spectra were obtained on a Waters Q-Tof premier spectrometer.

Plant Material The leaves of *K. flavida* were collected in Penang, Malaysia in 2006. The botanical identification was made by Prof. Kit-Lam Chan, School of Pharmaceutical Sciences, University Sains Malaysia. A voucher specimen is deposited at the Herbarium of University Sains Malaysia, Malaysia.

Table 2. ^{13}C NMR Data [δ_C] of Flavisiamines A (**1**) ~ D (**4**) in CD_3OD.

Position	**1**	**2**	**3**	**4**
2	76.0	75.8	76.7	76.6
3	45.3	47.5	45.1	45.3
5	53.6	53.9	53.6	53.5
6	54.1	54.1	54.3	54.2
7	60.4	60.2	60.7	60.2
8	129.6	130.0	130.8	125.9
9	124.0	124.1	116.4	117.2
10	111.9	120.8	122.0	101.0
11	131.2	131.1	113.5	151.5
12	120.8	111.8	147.5	133.6
13	150.2	150.2	140.0	130.8
14	24.0	25.2	24.1	24.0
15	67.7	71.1	67.9	67.7
16	205.6	206.7	206.0	205.4
17	43.8	40.1	43.8	43.7
18	27.4	27.3	27.4	27.3
19	32.0	31.8	32.1	32.0
20	39.6	40.1	39.6	39.6
21	66.1	70.7	66.0	66.0
22	174.3	174.3	174.6	174.1
23	52.8	52.8	52.9	52.9
OMe			56.3	
OCH_2O				102.8

Extraction and Isolation The leaves of *K. flavida* (200 g) were crushed and extracted with MeOH. The MeOH extract was treated with 3% tartaric acid (pH 2) and then partitioned with EtOAc. The aqueous layer was treated with saturated Na_2CO_3 (aq) to pH 10 and extracted with $CHCl_3$ to give alkaloidal fraction (660 mg). The alkaloidal fraction was purified by a SiO_2 column ($CHCl_3$/MeOH 1:0 → 0:1). The fractions eluted with $CHCl_3$/MeOH (10:1) were purified with ODS HPLC (YMC ODS-A A-302, YMC Co., Ltd., 10 x 250 mm; eluent, MeOH/H_2O/CF_3CO_2H, 10:90:0.05 → 25:75:0.05; flow rate, 1 mL/min; UV detection at 210 nm) to afford flavisiamine A (**1**, t_R 8.7 min, 1.1mg, 0.010%), flavisiamine B (**2**, t_R 7.9 min, 0.8 mg, 0.007%), flavisiamine C (**3**, t_R 14.9 min, 0.9mg, 0.008%), and flavisiamine D (**4**, t_R 11.9 min, 1.2 mg, 0.011%), together with five known alkaloids, *N*1-decarbomethoxychanofruticosinate,[5] methyl 11,12-methylenedioxy-*N*1-decarbomethoxy-chanofruticosinate,[5] methyl 11,12-methylenedioxy-*N*1-decarbomethoxy $\Delta^{14,15}$-chanofruticosinate,[5] methyl 12-methoxydecarbomethoxychanofruticosinate,[4a] oxychanofruticosinate.[4a]

Flavisiamine A (1): colorless amorphous solid; $[\alpha]_D^{27}$ +40 (*c* 0.7, MeOH); IR (KBr) ν_{max} 3346, 2943, 1732, 1676, 1198, and 755 cm^{-1}; UV (MeOH) λ_{max} 205 (ε 10600), 240 (2650), and 295 nm (1100); ^1H and

^{13}C NMR, see Table 1; ESIMS *m/z* 369 $(M+H)^+$; HRESIMS (*m/z* 369.1818 $[(M+H)^+$, calcd for $C_{21}H_{25}N_2O_4$, 369.1814].

Flavisiamine B (2): colorless amorphous solid; $[\alpha]_D^{27}$ +58 (*c* 0.7, MeOH); IR (KBr) ν_{max} 3380, 2930, 1730, 1680, 1197, and 760 cm^{-1}; UV (MeOH) λ_{max} 203 (ε 10000), 240 (2700), and 295 nm (1000);^{1}H and ^{13}C NMR, see Table 1; ESIMS *m/z* 369 $(M+H)^+$; HRESIMS (*m/z* 369.1812 $[(M+H)^+$, calcd for $C_{21}H_{25}N_2O_4$, 369.1814].

Flavisiamine C (3): colorless amorphous solid; $[\alpha]_D^{27}$ +32 (*c* 0.7, MeOH); IR (KBr) ν_{max} 3360, 2928, 1732, 1676, 1200, and 757 cm^{-1}; UV (MeOH) λ_{max} 210 (ε 9700), 245 (1900), and 295 nm (800);^{1}H and ^{13}C NMR, see Table 1; ESIMS *m/z* 399. $(M+H)^+$; HRESIMS (*m/z* 399.1917 $[(M+H)^+$, calcd for $C_{22}H_{27}N_2O_5$, 399.1920].

Flavisiamine D (4): colorless amorphous solid; $[\alpha]_D^{27}$ +35 (*c* 0.7, MeOH); IR (KBr) ν_{max} 3342, 2925, 1736, 1680, 1251, and 1201 cm^{-1}; UV (MeOH) λ_{max} 220 (ε 10200) and 295 nm (500); ^{1}H and ^{13}C NMR, see Table 1; ESIMS *m/z* 413 $(M+H)^+$; HRESIMS (*m/z* 413.1690 $[(M+H)^+$, calcd for $C_{22}H_{25}N_2O_6$, 413.1713].

ACKNOWLEDGMENTS

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