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FLAVISIAMINES E - F AND FRUTICOSIAMINE A, NEW METHYL CHANOFRUTICOSINATE- AND ASPIDOFRACTININE-TYPE INDOLE ALKALOIDS, FROM TWO SPECIES OF *KOPSIA*

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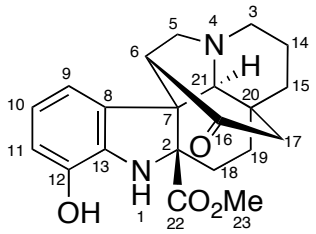
Abstract – Two new methyl chanofruticosinate-type indole alkaloids, flavisiamines E (**1**) and F (**2**), and an aspidofractinine-type indole alkaloid, fruticosiamine A (**3**), have been isolated from *Kopsia flavida* and *K. fruticosa*, respectively and their structures were elucidated by NMR spectral analysis using 2D techniques.

INTRODUCTION

Monoterpenoid indole alkaloids are a group of natural products with unique ring systems, which have attracted interest from structural and biological points of view.[1] In our research for structurally unique and biologically interesting indole alkaloids, we have previously isolated quaternary indole alkaloids, subincanadines from barks of *Aspidosperma subincanum* (Apocynaceae) and a bisindole alkaloid, huncaniterine A from the bark of *Hunteria zeylanica* (Apocynaceae).[2] The genus *Kopsia* and *Alstonia* (Apocynaceae), which are widely distributed throughout tropical Asia, are noted for producing variety of monoterpenoid 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 ~ D.[5] In our continuing search for structurally interesting alkaloids from tropical plants in Malaysia, two new methyl chanofruticosinate-type indole alkaloids, flavisiamines E (**1**) and F (**2**), and an aspidofractinine-type indole alkaloid, fruticosiamine A (**3**), have been isolated from the leaves of *K. flavida* and *K. fruticosa*, respectively. In this paper, we report the isolation and structure elucidation of flavisiamines E (**1**) and F (**2**), and fruticosiamine A (**3**).

The leaves of *K. flavida* and *K. fruticosa* 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 from *K. flavida* 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 (MeOH/H_2O/CF_3CO_2H, 10:90:0.05→25:75:0.05) to afford flavisiamine E (**1**, 0.018 %) together with flavisiamines A – D.[5] $CHCl_3$/MeOH (100:1) eluted fractions were purified by C_{18} HPLC (MeOH/H_2O/CF_3CO_2H, 15:75:0.05) to afford flavisiamine F (**2**, 0.006%) and prunifoline C.[6] $CHCl_3$-solubule materials from *K. fruticosa* were subjected to a silica gel column ($CHCl_3$/MeOH 1:0 → 0:1), and $CHCl_3$/MeOH (19:1) eluted fractions were purified by C_{18} HPLC (MeOH/H_2O/CF_3CO_2H, 25:75:0.05) to afford fruticosiamine A (**3**, 0.001%) together with four known compounds, fruticosamine,[10] fruticosine,[10] kopsine,[11] and methyl *N*1-decarbomethoxychanofruticosinate.[7]



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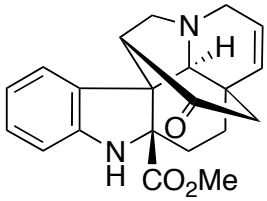

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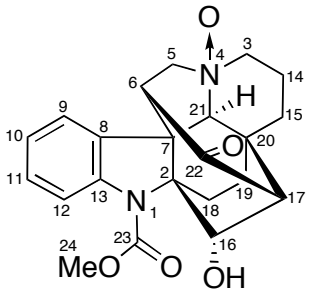

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RESULTS AND DISCUSSION

Flavisiamine E (**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* 369.1812, $(M+H)^+$ Δ -0.2 mDa]. IR spectrum suggested the presence of OH (3380 cm^{-1}) and carbonyl (1690 cm^{-1}) groups. The ^{13}C NMR (Table 1) spectrum of **1** disclosed twenty-one carbon signals due to one carbonyl (δ_C 206.2), one ester carbonyl (δ_C 174.8), three sp^2 quaternary carbons (δ_C 130.8, 137.4, and 144.7), three sp^3 quaternary carbons (δ_C 35.2, 60.4, and 76.7), three sp^2 methines (δ_C 115.1, 117.8, and 122.2), two sp^3 methines (δ_C 53.8 and 71.2), seven sp^3 methylenes (δ_C 16.5, 27.5, 32.2, 36.0, 43.5, 50.1, and 54.1), and one methyl (δ_C 52.9) 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-11, and C-18 to C-19 (Figure 1). HMBC correlations of H-9 (δ_H 6.78) to C-7 (δ_C 60.4), H-10 (δ_H 6.74) to C-12 (δ_C 137.4), H-21 (δ_H 3.57) to C-2 (δ_C 76.7), C-7, C-8 (δ_C 130.8), and C-19

(δ_C 36.0), and H_2-18 (δ_H 2.16 and 1.87) to C-7 revealed the presence of a hexahydrocarbazole ring with a hydroxyl at C-12 (C-2, C-18 - C-21, C-7-C-13, and *N*-1). A cross peak of H-21 to C-5 (δ_C 54.1) 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) was deduced from the HMBC correlations of H-21 to C-3 (δ_C 50.1) and C-15 (δ_C 32.2). HMBC correlations of H-17a (δ_H 3.10) to C-16 (δ_C 206.2) and C-20 (δ_C 35.2) 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 3.57) to C-22 (δ_C 174.8) were implied methoxy carbonyl group connected at C-2. Thus, the structure of flavisiamine E was elucidated to be **1**, whose NMR data were similar to those of methyl *N*1-decarbomethoxychanofruticosinate with methyl chanofruticosinate skeleton.[7] The relative stereochemistry of **1** was elucidated by ROESY correlations as shown in Figure 2.

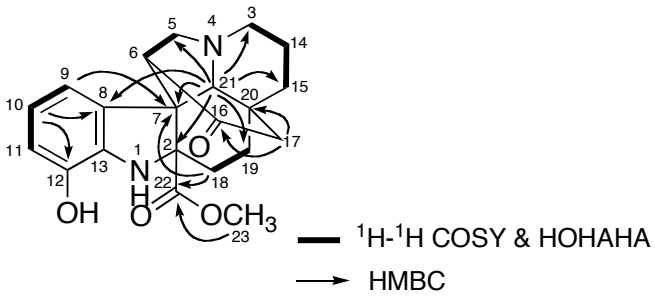

Figure 1. Selected 2D NMR correlations for flavisiamine E (**1**)

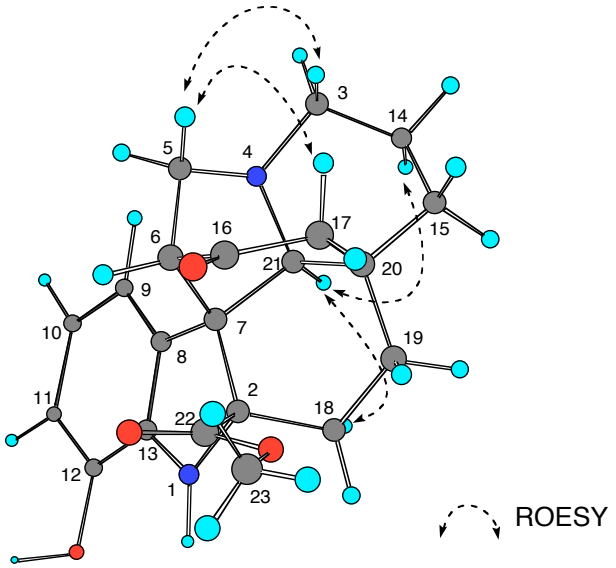

Figure 2. Selected ROESY correlations for flavisiamine E (**1**)

Flavisiamine F (**2**) showed the pseudomolecular ion peak at *m/z* 351 $(M+H)^+$ in ESIMS, and the molecular formula was established to be $C_{21}H_{22}N_2O_3$ by HRESIMS [**2**: *m/z* 351.1694 $(M+H)^+$ Δ -1.5 mDa]. Compared the NMR data of **2** with those of methyl 11,12-methylenedioxy-*N*1-decarbomethoxy $\Delta^{14,15}$-chanofruticosinate with 1,2,3,6-tetrahydropyridine ring,[8] the similar chemical shift pattern except for a benzene ring was observed. ^{1}H-^{1}H COSY spectrum of **2** indicated connectivities of C-9 - C-12 in the benzene ring. Thus, flavisiamine F was assigned as **2**.

Fruticosiamine A (**3**) showed the pseudomolecular ion peak at *m/z* 397 $(M+H)^+$ in ESIMS, and molecular formula, $C_{22}H_{24}N_2O_5$, was established by HRESIMS [*m/z* 397.1763, $(M+H)^+$ Δ ±0.0 mDa]. The IR spectrum suggested the presence of OH (3380 cm^{-1}), and carbonyl groups (1700 and 1680 cm^{-1}). The structure of **3** was elucidated by ^{1}H and ^{13}C NMR spectra and 2D NMR analysis (^{1}H-^{1}H COSY, HSQC, and HMBC spectra). The ^{13}C NMR spectrum of **3** (Table 1) disclosed twenty two carbon signals due to two carbonyls (δ_C 155.2 and 208.2), two sp^2 quaternary carbons (δ_C 126.0 and 141.4), three sp^3 quaternary carbons (δ_C 37.4, 61.7, and 71.2), four sp^2 methines (δ_C 116.5, 125.3, 127.8, and 130.5), four sp^3 methines (δ_C 53.6, 60.0, 70.8, and 82.8), six sp^3 methylenes (δ_C 18.9, 21.9, 30.9, 32.3, 68.0, and 73.3), and one methyl (δ_C 54.1) attached to an oxygen atom. Proton and carbon signals for **3** were assigned by detailed analysis of the HSQC spectrum. Based on the correlations of ^{1}H-^{1}H COSY and HMBC spectra, **3** was aspidofractinine-type skeleton as the same as fruticosamine.[10] Low field chemical shifts at C-3, C-5, and C-21 around *N*-4 atom [C-3 (δ_H 2.40 δ_C 48.2 → δ_H 4.18 δ_C 68.0), C-5 (δ_H 3.56 2.85 δ_C 54.2 → δ_H 4.68 4.20, δ_C 73.3), and C-21 (δ_H 4.82 δ_C 82.8)] on comparison with those in fruticosamine, suggested that **3** was *N*-oxide form at *N*-4. Oxidation of fruticosamine with *m*-chloroperoxybenzoic acid (*m*-CPBA) afforded the *N*-oxide derivative, whose spectral data and the $[\alpha]_D$ value were identical with those of natural fruticosiamine A (**3**). Thus, fruticosiamine A (**3**) was concluded to be the *N*-oxide form of fruticosamine.

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 $^nJ_{CH}$=8Hz. Positive-mode ESI mass spectra were obtained on a Waters Q-Tof premier spectrometer.

Plant Material The leaves of *K. flavida* and *K. fruticosa* 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.

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 flavisiamines E (**1**, t_R 9.8 min, 1.9 mg, 0.018%) together with flavisiamines A – D.[5] The fractions eluted with $CHCl_3$/MeOH (100:1) were purified by C_{18} HPLC (MeOH/H_2O/CF_3CO_2H, 15:75:0.05) to afford flavisiamine F (**2**, t_R 5.8 min, 0.7 mg, 0.006%) and prunifoline C.[6]

Table 1. ^{1}H NMR Data [δ_H (J, Hz)] of Flavisiamines E (**1**) ~ F (**2**) in CD_3OD and Fruticosiamine A (**3**) in $CDCl_3$.

Position	**1**	**2**	**3**
3a	3.47 (dd, 13.5, 4.5)	4.21 (m)	4.18 (m)
3b	3.44 (dd, 13.5, 5.6)	4.03 (m)	
5a	4.43 (dd, 14.4, 7.0)	4.85 (m)	4.68 (dd, 13.6 6.6)
5b	3.79 (d, 14.4)	3.75 (m)	4.20 (d, 13.8)
6	3.55 (d, 7.0)	3.59 (m)	2.91 (d, 6.6)
9	6.78 (d, 8.4)	7.26 (m)	8.35 (d, 7.2)
10	6.74 (t, 8.4)	6.82 (m)	7.17 (t, 7.2)
11	6.74 (d, 8.4)	7.17 (m)	7.36 (t, 7.2)
12			7.65 (d, 7.2)
14a	2.30 (m)	5.80 (m)	1.89 (m)
14b	1.87 (m)		1.61 (m)
15a	1.66 (m)	6.19 (m)	1.86 (m)
15b	1.56 (dt, 12.6, 3.5)		
16			4.14 (s)
17a	3.10 (d, 19.6)	2.84 (d, 18.5)	2.52 (s)
17b	2.34 (d, 19.6)	2.61 (d, 18.5)	
18a	2.16 (dt, 14.9, 4.1)	2.14 (dd, 14.8, 3.9)	2.43 (dd, 13.6 9.6)
18b	1.87 (m)	1.84 (dt, 14.8, 4.4)	1.82 (m)
19a	2.00 (dt, 12.9, 4.1)	2.06 (dt, 13.5, 4.4)	2.65 (dd, 15.6 9.6)
19b	1.67 (m)	1.95 (dd, 13.5, 3.9)	1.73 (m)
21	3.57 (brs)	3.58 (brs)	4.82 (s)
23	3.57 (s)	3.57 (s)	
24			3.97 (s)
OH			5.95 (brs)

Table 2. ^{13}C NMR Data [δ_C] of Flavisiamines E (**1**) ~ F (**2**) in CD_3OD and Fruticosiamine A (**3**) in $CDCl_3$.

Position	**1**	**2**	**3**
2	76.7	76.4	71.2
3	50.1	52.3	68.0
5	54.1	61.3	73.3
6	53.8	53.8	53.6
7	60.4	59.7	61.7
8	130.8	130.3	126.0
9	115.1	124.0	127.8
10	122.2	120.8	125.3
11	117.8	130.9	130.5
12	137.4	112.0	116.5
13	144.7	150.4	141.4
14	16.5	122.5	18.9
15	32.2	137.1	32.3
16	206.2	205.8	70.8
17	43.5	46.5	60.0
18	27.5	27.1	21.9
19	36.0	33.4	30.9
20	35.2	36.0	37.4
21	71.2	70.2	82.8
22	174.8	174.3	208.2
23	52.9	52.8	155.2
24			54.1

The leaves of *K. fruticosa* (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 (823 mg). The portion of alkaloidal fraction (205 mg) was purified by a SiO_2 column ($CHCl_3$/MeOH 1:0 → 0:1). The fractions eluted with $CHCl_3$/MeOH (19:1) were purified with C_{18} HPLC (YMC ODS-A A-302, YMC Co., Ltd., 10 x 250 mm; eluent, MeOH/H_2O/CF_3CO_2H, 25:75:0.05; flow rate, 1 mL/min; UV detection at 210 nm) to afford fruticosiamine A (**3**, t_R 15.8 min, 0.5 mg, 0.001%) together with fruticosamine,[10] fruticosine,[10] kopsine,[11] and methyl *N*1-decarbomethoxychanofruticosinate.[7]

Flavisiamine E (1): colorless amorphous solid; $[\alpha]_D^{27}$ +38 (*c* 0.7, MeOH); IR (KBr) ν_{max} 3380, 2930, 1730, 1680, 1197, and 1127 cm^{-1}; UV (MeOH) λ_{max} 208 (ε 9300) and 290 nm (800); ^1H 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 F (2): colorless amorphous solid; $[\alpha]_D^{27}$ +90 (*c* 0.7, MeOH); IR (KBr) ν_{max} 3380, 2930, 1730, 1680, 1197, and 1127 cm^{-1}; UV (MeOH) λ_{max} 205 (ε10000), 240 (2700), and 295 nm (1000); ^1H and ^{13}C NMR, see Table 1; ESIMS *m/z* 351 $(M+H)^+$; HRESIMS (*m/z* 351.1694 $[(M+H)^+$, calcd for $C_{21}H_{23}N_2O_3$, 351.1709].

Fruticosiamine A (3): colorless amorphous solid; $[\alpha]_D^{27}$ +40 (*c* 1.0, $CHCl_3$); IR (KBr) ν_{max} 3380, 2930, 1700, 1680, 1197, and 1127 cm^{-1}; UV (MeOH) λ_{max} 208 (ε 10000), 240 (5000), and 280 nm (800); ^1H and ^{13}C NMR, see Table 1; ESIMS *m/z* 397 $(M+H)^+$; HRESIMS (*m/z* 397.1763 $[(M+H)^+$, calcd for $C_{22}H_{25}N_2O_5$, 397.1763].

Oxidation of fruticosamine. *m*-Chloroperoxybenzoic acid (2 equv.) was added to a stirred solution of fruticosamine (1.0 mg) in $CHCl_3$ (0.5 mL) at rt. The mixture was stirred at rt for 3 h, and washed with 20% Na_2CO_3 (aq) (10 mL) and H_2O (10 mL), and concentrated to give a pale yellow oil (1.1 mg). The residue was subjected to a silica gel column chromatography ($CHCl_3$/MeOH, 15:1) to give the *N*-oxide derivative (0.9 mg), whose spectral data and $[\alpha]_D$ value were identical with those of fruticosiamine A (**3**).

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