Two New Bis-alkaloids from the Aerial Part of *Piper flaviflorum*

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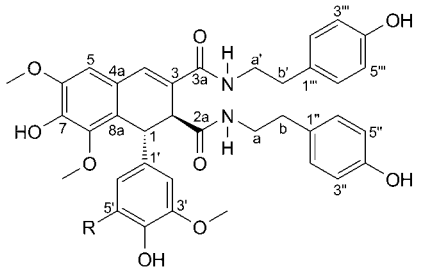
Two new bis-alkaloids, flavifloramides A (**1**) and B (**2**), as well as two known alkaloids, *N-trans*-feruloyltyramine (**3**) and paprazine (**4**), were isolated from the aerial part of *Piper flaviflorum*. The structures of the new compounds were elucidated by spectroscopic analyses, including 2D-NMR techniques.

Introduction. – Plants of the *Piper* genus are well-known as rich sources of a variety of alkaloids, which have been reported to possess versatile beneficial pharmacological activities, such as anti-inflammatory, antinociceptive, anticancer, and antidepressant properties [1–9]. In the course of searching for novel bioactive components derived from the plants of the *Piper* genus, two new bis-alkaloids, also named lignanamides, *i.e.*, flavifloramides A[2]) (**1**) and B[2]) (**2**), as well as two known alkaloids, *N-trans*-feruloyltyramine (=(2*E*)-3-(4-hydroxy-3-methoxyphenyl)-*N*-[2-(4-hydroxyphenyl)-ethyl]prop-2-enamide; **3**) and paprazine (=(2*E*)-3-(4-hydroxyphenyl)-*N*-[2-(4-hydroxyphenyl)ethyl]prop-2-enamide; **4**), were isolated from the aerial part of *Piper flaviflorum*, an indigenous species in southern China. This article describes the isolation and structure elucidation of the new compounds.

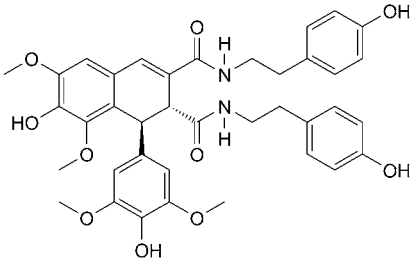
Results and Discussion. – Repeated column chromatography of the CH_2Cl_2 extract from the aerial part of *P. flaviflorum* afforded compounds **1**–**4**.

Flavifloramide A (**1**), which was obtained as an amorphous white powder, had the molecular formula $C_{37}H_{38}N_2O_{10}$ with 18 degrees of unsaturation, deduced by HR-ESI-MS (*m*/*z* 671.2524 ($[M+H]^+$)). The IR spectrum showed absorption bands for amide C=O groups at 1647 and 1613 cm^{-1} and for OH groups at 3356 cm^{-1}. The ^{1}H-NMR spectrum (*Table 1*) exhibited eight aromatic H-atoms giving rise to two pairs of *d*s (δ(H) 6.66 and 6.94 (2*d*, *J* = 8.4 Hz, 2 H each), and δ(H) 6.63 and 6.80 (2*d*, *J* = 8.4 Hz, 2 H each)). Another two pairs of correlated ^{1}H-NMR signals (δ(H) 3.16–3.18 and 3.33–3.35 (2*m*, 1 H each), δ(H) 2.51 and 2.52 (2*t*, 1 H each), and δ(H) 3.36–3.37 (*m*, 2 H), and 2.67 (*t*, 2 H)) together with the IR spectrum (1647 and 1613 cm^{-1}) suggested the presence of two acylated tyramine (=4-(2-aminoethyl)phenol) moieties in the molecule. Moreover, four aromatic H-atoms appeared at δ(H) 6.03 and 6.32 (2*d*, *J* = 1.8 Hz, 1 H each) and δ(H) 6.76 and 7.26 (2*s*, 1 H each), together with three MeO

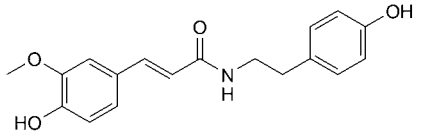
[1]) These two authors have contributed equally to this work.
[2]) Arbitrary atom numbering; for systematic names, see *Exper. Part.*



1[2]) R = OH
5[2]) R = MeO



2[2])



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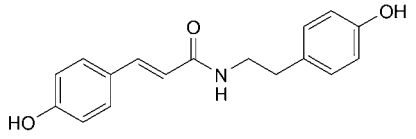

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Table 1. *^{1}H-NMR Data* (600 MHz, CD_3OD, at 27°) *of* **1**, **2**, *and* **5**. δ in ppm, J in Hz.

H-Atom[2])	**1**	**5**	**2**
H–C(1)	4.79 (br. *s*)	5.02 (br. *s*)	4.84 (br. *s*)
H–C(2)	3.66 (*d*, $J = 1.2$)	3.70 (*s*, 1 H)	3.70 (*d*, $J = 1.8$, 1 H)
H–C(4)	7.26 (*s*)	7.20 (*s*)	7.28 (*s*)
H–C(5)	6.76 (*s*)	6.68 (*s*)	6.76 (*s*)
H–C(2′,6′)	6.32, 6.03 (2*d*, $J = 1.8$ each 1 H)	6.24 (*s*, 2 H)	6.33 (*s*, 2 H)
CH_2(a)	3.33–3.35, 3.16–3.18 (2*m*, each 1 H)	3.38 (*m*)	3.36–3.42, 3.17–3.21 (2*m*, each 1 H)
CH_2(a′)	3.36–3.37 (*m*)	3.26 (*m*)	3.33–3.35 (*m*)
CH_2(b)	2.52, 2.51 (2*t*, each 1 H)	2.53, 2.52 (2*t*, each 1 H)	2.53, 2.52 (2*t*, each 1 H)
CH_2(b′)	2.67 (*t*)	2.67 (*t*)	2.67 (*t*)
H–C(2″,6″)	6.94 (*d*, $J = 8.4$, 2 H)	6.73 (*d*, $J = 8.5$, 2 H)	6.92 (*d*, $J = 9.0$, 2 H)
H–C(2‴,6‴)	6.80 (*d*, $J = 8.4$, 2 H)	6.84 (*d*, $J = 8.5$, 2 H)	6.81 (*d*, $J = 8.4$, 2 H)
H–C(3″,5″)	6.66 (*d*, $J = 8.4$, 2 H)	6.54 (*d*, $J = 8.5$, 2 H)	6.64 (*d*, $J = 9.0$, 2 H)
H–C(3‴,5‴)	6.63 (*d*, $J = 8.4$, 2 H)	6.56 (*d*, $J = 8.5$, 2 H)	6.63 (*d*, $J = 8.4$, 2 H)
MeO–C(3′)	3.73 (*s*)	3.60 (*s*)	3.68 (*s*)
MeO–C(5′)	–	3.60 (*s*)	3.68 (*s*)
MeO–C(6)	3.91 (*s*)	3.82 (*s*)	3.90 (*s*)
MeO–C(8)	3.56 (*s*)	3.48 (*s*)	3.57 (*s*)

groups at δ(H) 3.56, 3.73, and 3.91 (3*s*). HMBC Cross-peaks suggested to position the three MeO groups at C(6), C(8), and C(3′) (*Fig.*). As confirmed by the DEPT experiment, the downfield signals at δ(C) 170.0 and 174.0 corresponded to two C=O groups; moreover, the signals of twenty-six aromatic C-atoms (δ(C) 104.1, 109.2, 109.4, 116.3, 124.4, 125.3, 127.0, 130.7, 130.8, 131.2, 131.4, 133.7, 135.2, 135.4, 143.1, 146.1, 147.0, 149.2, 149.5, 156.7, and 156.8) three MeO groups (δ(C) 56.7, 56.8, and 60.8), four CH_2 groups (δ(C) 35.4, 35.6, 42.4, and 42.8), and two aliphatic CH groups (δ(C) 41.4 and 50.3) were also present (*Table 2*).

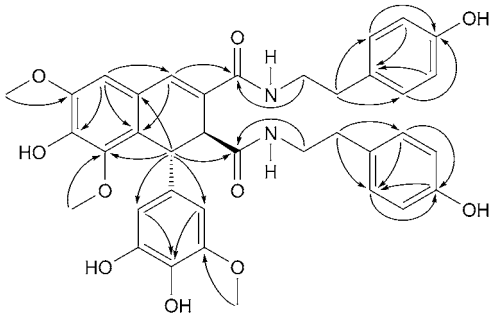

Figure. *Key HMBCs of* **1**

Table 2. *^{13}C-NMR Data* (150 MHz, CD_3OD, 27°) *of* **1**, **2**, *and* **5**. δ in ppm.

C-Atom[2])	**1**	**5**	**2**
C(1)	41.4 (*d*)	41.6 (*d*)	41.6 (*d*)
C(2)	50.3 (*d*)	49.2 (*d*)	50.2 (*d*)
C(3)	127.0 (*s*)	127.1 (*s*)	127.2 (*s*)
C(4)	135.2 (*d*)	135.1 (*d*)	135.2 (*d*)
C(5)	109.2 (*d*)	109.1 (*d*)	109.1 (*d*)
C(6)	149.2 (*s*)	149.2 (*s*)	149.2 (*s*)
C(7)	143.1 (*s*)	143.1 (*s*)	143.1 (*s*)
C(8)	147.0 (*s*)	146.9 (*s*)	147.0 (*s*)
C(2a)	174.0 (*s*)	174.0 (*s*)	174.0 (*s*)
C(3a)	170.0 (*s*)	170.0 (*s*)	170.0 (*s*)
C(4a)	124.4 (*s*)	124.3 (*s*)	124.3 (*s*)
C(8a)	125.3 (*s*)	125.2 (*s*)	125.2 (*s*)
C(1′)	135.4 (*s*)	135.3 (*s*)	135.3 (*s*)
C(2′)	104.1 (*d*)	106.0 (*d*)	106.0 (*d*)
C(3′)	149.5 (*s*)	149.0 (*s*)	149.0 (*s*)
C(4′)	133.7 (*s*)	135.3 (*s*)	135.1 (*s*)
C(5′)	146.1 (*s*)	149.0 (*s*)	149.0 (*s*)
C(6′)	109.4 (*d*)	106.0 (*d*)	106.0 (*d*)
C(1″)	131.2 (*s*)	131.1 (*s*)	131.1 (*s*)
C(2″,6″)	130.8 (*d*)	130.7 (*d*)	130.8 (*d*)
C(3″,5″)	116.3 (*d*)	116.2 (*d*)	116.2 (*d*)
C(4″)	156.8 (*s*)	156.8 (*s*)	156.8 (*s*)
C(1‴)	131.4 (*s*)	131.3 (*s*)	131.4 (*s*)
C(2‴,6‴)	130.7 (*d*)	130.8 (*d*)	130.7 (*d*)
C(3‴,5‴)	116.3 (*d*)	116.2 (*d*)	116.2 (*d*)
C(4‴)	156.7 (*s*)	156.8 (*s*)	156.8 (*s*)
C(a)	42.8 (*t*)	42.4 (*t*)	42.8 (*t*)
C(a′)	42.4 (*t*)	42.8 (*t*)	42.4 (*t*)
C(b)	35.4 (*t*)	35.4 (*t*)	35.4 (*t*)
C(b′)	35.6 (*t*)	35.6 (*t*)	35.6 (*t*)
*Me*O–C(3′)	56.7 (*q*)	56.7 (*q*)	56.7 (*q*)
*Me*O–C(5′)	–	56.7 (*q*)	56.7 (*q*)
*Me*O–C(6)	56.8 (*q*)	56.8 (*q*)	56.8 (*q*)
*Me*O–C(8)	60.8 (*q*)	60.8 (*q*)	60.8 (*q*)

The NMR spectra of **1** were analogous to those of the known compound **5** [10] (*Table 2*), except for the disappearance of the signal corresponding to an MeO group. The relative configuration at C(1) and C(2) could be assigned in analogy with that of **5**, which was confirmed by the negative optical rotation value and the small coupling constant between H–C(l) (δ(H) 4.79 (br. *s*) and H–C(2) (δ(H) 3.66 (*d*, $J = 1.2$ Hz)), suggesting a relative *trans* configuration between H–C(1) (β-oriented) and H–C(2) (α-oriented) [11]. Finally, the structure of **1** was elucidated as the 5′-*O*-demethyl derivative of **5**, and it was named flavifloramide A[2]).

Flavifloramide B (**2**), was obtained as an amorphous white powder which possessed a molecular formula $C_{38}H_{40}N_2O_{10}$ on the basis of its HR-ESI-MS (*m*/*z* 685.2683 ($[M+H]^+$)), indicating 18 degrees of unsaturation. The IR spectrum also indicated the presence of amide C=O groups (1653 and 1613 cm^{-1}) and OH groups (3290 cm^{-1}). According to the molecular formula and NMR data, **2** was determined to possess the same general pattern as **5**, differing only in the ^{1}H-NMR signals of H–C(1) (δ(H) 4.84 (br. *s*) in **2** and 5.02 (br. *s*) in **5**; *Table 1*). Moreover, opposite optical-rotation values, *i.e.*, $[\alpha]_D^{25} = +19$ ($c = 0.10$, MeOH) for **2** and $[\alpha]_D^{25} = -20$ ($c = 0.062$, MeOH) for **5** [10], indicated α- and β-orientation of H–C(1) and H–C(2) in **2**, respectively, opposite to those in **1** and **5** (relative configurations). The structure of **2** was, therefore, elucidated as shown, and it was named flavifloramide B[2]).

The known alkaloid compounds were identified as *N-trans*-feruloyltyramine (**3**) and paprazine (**4**) by comparing their ^{1}H- and ^{13}C-NMR data with those reported [12–14]. From the structures of **3** and **4**, it could be deduced that they acted as molecular units of **1** and **2** in the course of the secondary-metabolite biosynthesis in the plant [15].

Experimental Part

General. Anal. TLC: silica-gel plates (*Yantai Institute of Chemical Technology*), petroleum ether/AcOEt 1:1 as eluent; visualization under UV light, and by spraying with 7% aq. H_2SO_4 soln., followed by heating. Column chromatography (CC): silica gel (SiO_2, 200–300 or 300–400 mesh; *Qingdao Marine Chemical Factory*). Optical rotations: *Jasco-P-1020* spectropolarimeter. UV Spectra: *Shimadzu-UV-260* spectrophotometer; anh. MeOH solns.; λ_{max} (log ε) in nm. IR Spectra: *Avatar-360-ESP* spectrophotometer (*Thermo Nicolet*); KBr pellets; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *DRX-600* spectrometer; CD_3OD solns.; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: *Bruker Apex-7.0-Tesla* FT-MS apparatus; in *m*/*z*.

Plant Material. The aerial parts of *Piper flaviflorum* were collected in Xishuangbanna, Yunnan Province, P. R. China, in May 2011. A voucher specimen (#201104) was deposited with the Herbarium of Materia Medica, School of Pharmacy, Second Military Medical University, Shanghai, P. R. China.

Extraction and Isolation. The air-dried aerial part (10 kg) of *P. flaviflorum* was extracted exhaustively with 80% aq. EtOH at r.t. The EtOH extract was concentrated to yield a semi-solid (700 g), which was suspended in H_2O (700 ml), and extracted with CH_2Cl_2 (3×500 ml). The combined org. phase was concentrated to yield a residue (100 g), part of which (90 g) was subjected to CC (SiO_2 (1 kg), petroleum ether/AcOEt gradient): *Fractions 1–7. Fr. 6,* eluted with petroleum ether/AcOEt 1:1, was subjected to repeated CC (SiO_2; petroleum ether/AcOEt 3:1), and then to prep. PTLC (petroleum ether/AcOEt 1:1): **1** (6.4 mg) and **2** (10 mg). *Fr. 5* eluted with petroleum ether/AcOEt 1:1, was subjected to repeated CC (SiO_2, petroleum ether/AcOEt 2:1): **3** (100 mg) and **4** (160 mg).

Flavifloramide A (= rel-*(1*R,*2*S*)-1-(3,4-Dihydroxy-5-methoxyphenyl)-1,2-dihydro-7-hydroxy*-N^2,N^3-*bis[2-(4-hydroxyphenyl)ethyl]-6,8-dimethoxynaphthalene-2,3-dicarboxamide*; **1**): Amorphous powder. $[\alpha]_D^{25} = -13$ ($c = 0.10$, MeOH). UV (MeOH): 213 (4.82), 245 (4.44), 293 (4.03), 330 (4.16). IR (KBr):

3356, 2936, 2843, 1647, 1613, 1514, 1462, 1237, 1087, 831. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 671.2524 ($[M+H]^+$, $C_{37}H_{39}N_2O_{10}^+$; calc. 671.2526).

Flavifloramide B (= rel-*(1*R,2S*)-1,2-Dihydro-7-hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)*-N^2,N^3-*bis[2-(4-hydroxyphenyl)ethyl]-6,8-dimethoxynaphthalene-2,3-dicarboxamide*; **2**): Amorphous powder. $[\alpha]_D^{25} = +19$ ($c = 0.10$, MeOH). UV (MeOH): 215 (4.80), 246 (4.41), 294 (4.01), 324 (4.15). IR (KBr): 3290, 2923, 2851, 1707, 1653, 1613, 1514, 1494, 1462, 1217, 1113, 831. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 685.2683 ($[M+H]^+$, $C_{38}H_{41}N_2O_{10}^+$; calc. 685.2685).

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