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Norsecurinamines A and B, two norsecurinine-derived alkaloid dimers from the fruits of *Flueggea virosa*

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Norsecurinamines A and B, two norsecurinine-derived alkaloid dimers from the fruits of *Flueggea virosa*

Gui-Yang Wang^{a,b,†}, Ai-Ting Wang^{a,b,†}, Bing-Xin Zhao^c, Xue-Ping Lei^a, Dong-Mei Zhang^a, Ren-Wang Jiang^a, Ying Wang^{a,b,*}, Wen-Cai Ye^{a,b,*}

^aInstitute of Traditional Chinese Medicine & Natural Products, College of Pharmacy, Jinan University, Guangzhou 510632, People's Republic of China

^bJNU-HKUST Joint Laboratory for Neuroscience & Innovative Drug Research, College of Pharmacy, Jinan University, Guangzhou 510632, People's Republic of China

^cDepartment of Chemistry, Jinan University, Guangzhou 510632, People's Republic of China

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ABSTRACT

The phytochemical investigation on the fruits of *Flueggea virosa* resulted in the discovery of two new norsecurinine-derived alkaloid dimers, norsecurinamines A and B (**1** and **2**). Compound **1** is the first hetero-dimeric alkaloid possessing norsecurinine and donaxaridine units, and **2** represents the first example of *NH*-linked dimeric *Securinega* alkaloid. Their structures and absolute configuration were elucidated on the basis of extensive spectroscopic analysis as well as single-crystal X-ray diffraction method. A plausible biogenetic pathway of **1** was also proposed.

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The genus *Flueggea* belongs to family Euphorbiaceae, which consists of 12 species and is widely distributed in tropical to warm temperate regions^[1]. The plants of genus *Flueggea* are a rich source of *Securinega* alkaloids, a class of indolizidine alkaloids with unique bridged tetracyclic structures^[2]. Structurally, *Securinega* alkaloids feature a core skeleton including a butenolide moiety (ring D) and an azabicyclo [3.2.1] octane system (rings B and C). According to the size of ring A, this family could be classified into two subgroups: securinine-type (six-membered piperidine) and norsecurinine-type (five-membered pyrrolidine)^[3]. Some *Securinega* alkaloids showed significant biological activities on central nervous system and cytotoxicity^[4–9]. Due to their remarkable biological activities and intriguing molecular structural features, *Securinega* alkaloids have attracted great interest from both chemists and biologists.

The plant *Flueggea virosa* (Roxb. ex Willd.) Voigt, Chinese name “bai fan shu”, is a shrub widely distributed in eastern, southern, and southeastern regions of China^[1]. The whole plants of *F. virosa* were used as Chinese folk medicine for the treatment of rheumatism, pruritus, cephalic eczema, leucorrhoea, and injuries^[1]. In recent years, a series of novel norsecurinine-derived alkaloid oligomers linked via ether bond or C–C bond

had been reported from the twigs and leaves of *F. virosa*^[10–16]. As part of our ongoing efforts in searching structural unique and biologically interesting alkaloids from the plants of genus *Flueggea*^[17–20], we recently carried out the first phytochemical investigation on the fruits of *F. virosa*. As a result, two new *NH*-bridged norsecurinine-derived alkaloid dimers, norsecurinamines A and B (**1** and **2**), along with their putative biosynthetic precursors *N*-methyltryptamine (**3**) and norsecurinine (**4**), were isolated from the ripened fruits of the title plant. Compound **1** is the first hetero-dimeric alkaloid with norsecurinine and donaxaridine units linked through an amino group. Compound **2** is a symmetrical *Securinega* alkaloid dimer comprised of two molecules of norsecurinine-type alkaloids connected via an amino function, which is the first *NH*-linked norsecurinine-derived alkaloid oligomer. Herein, we describe the isolation, structural elucidation, and cytotoxic effects of **1** and **2**. Additionally, based on the isolation of its putative biosynthetic precursors, a plausible biosynthetic pathway of compound **1** is also proposed.

Norsecurinamine A (**1**) was obtained as colorless needles. The molecular formula of **1** was established as C₂₃H₂₇N₃O₄ from its HR-ESI-MS (*m/z* 410.2077 [M + H]⁺, calcd for C₂₃H₂₈N₃O₄;

* Corresponding author: Tel: 86-20-85220004, E-mail: chywc@aliyun.com (Wen-Cai Ye);

Tel: 86-20-85223553, E-mail: wangying_cpu@163.com (Ying Wang)

† These authors contributed equally to this work.

410.2074). The UV spectrum of **1** showed absorption maxima at 206, 256, and 294 nm. The IR spectrum implied the existence of hydroxyl group (3351 cm^{-1}), α,β -unsaturated γ -lactone ring (1763 and 1687 cm^{-1}), and aromatic ring (1581 and 1451 cm^{-1}) in **1**. The analysis of ^1H and ^{13}C NMR spectra revealed that **1** possessed an *ortho*-disubstituted benzene ring [δ_{H} 7.12 (1H, dd, $J = 7.6, 7.6\text{ Hz}$), 6.80 (1H, d, $J = 7.6\text{ Hz}$), 6.76 (1H, d, $J = 7.6\text{ Hz}$), and 6.58 (1H, dd, $J = 7.6, 7.6\text{ Hz}$); δ_{C} 130.2, 127.5, 117.5, and 113.8], an α,β -unsaturated γ -lactone ring [δ_{H} 5.70 (1H, d, $J = 1.9\text{ Hz}$); δ_{C} 175.7 ($\times 2$), 110.5, and 93.3], a *N*-methyl group [δ_{H} 2.96 (3H, s); δ_{C} 30.3], and a carbonyl group (δ_{C} 177.2). Comprehensive analysis of NMR data allowed the assignment of all ^1H and ^{13}C NMR signals of **1** (Table 1).

Figure 1. Structures of **1** and **2**

Table 1. NMR data of **1** (in CD_3OD , J in Hz)^a

No.	δ_{H}	δ_{C}	No.	δ_{H}	δ_{C}
2	3.16	68.4	1'	-	147.1
3	a 1.81	30.0	2'	-	126.2
	b 1.78		3'	6.80 d (7.6)	127.5
4	a 1.94 m	27.6	4'	6.58 dd (7.6, 7.6)	117.5
	b 1.71		5'	7.12 dd (7.6, 7.6)	130.2
5	a 3.32 m	58.5	6'	6.76 d (7.6)	113.8
	b 2.63 m		7'	-	177.2
7	3.17	66.0	8'	-	81.3
8	a 2.59 dd (11.3, 6.3)	33.7	9'	a 2.66	34.9
	b 1.56 d (11.3)			b 2.33 m	
9	-	93.7	10'	a 3.38	46.7
11	-	175.7		b 3.14	
12	5.70 d (1.9)	110.5	11'	2.96 s	30.3
13	-	175.7			
14	a 3.27	31.9			
	b 2.50 ddd (15.6, 10.1, 2.5)				
15	3.74 m	55.4			

^a Overlapped signals were reported without designating multiplicity.

The ^1H - ^1H COSY spectrum of **1** revealed the presence of four spin systems (C-2 to C-5, C-8 to C-14, C-3' to C-6', and C-9' to C-10'; Figure 2). In the HMBC spectrum, correlations between H-2 and C-5/C-7, between H₂-8 and C-2/C-13, as well as between H-12 and C-9/C-14 allowed the establishment of the planar structure of dihydronorsecurinine moiety (**1a**). Similarly, the HMBC correlations between H-3' and C-1'/C-8', between H-6' and C-2', between H-9' and C-2'/C-7', between H-10' and C-8'/C-7', as well as between H-11' and C-7'/C-10' established the backbone structure of donaxaridine moiety (**1b**). The NMR data of **1a** and **1b** moieties were very similar to those of dihydronorsecurinine^[21] and donaxaridine^[22], respectively. Moreover, the HMBC correlations between H-15 and C-1'

combined with the obvious downfield shift of C-15 (δ_{C} 55.4) and upfield shift of C-1' (δ_{C} 147.1) suggested that the **1a** and **1b** were connected through C-15 and C-1' via an amino group.

Figure 2. Key ^1H - ^1H COSY and HMBC correlations of **1** and **2**

The NOESY spectrum of **1** showed the correlations between H-2 and H-5b, suggesting that H-2 and H-5b had the same orientation. On the contrary, NOE correlations between H-7 and H-5a/H-15, as well as between H-8a and H-15 suggested that these protons were located in the same orientation of the molecule (Figure 3). However, the NOESY data did not provide enough information to establish the stereochemistry of C-8'. Fortunately, crystals suitable for a single-crystal X-ray diffraction experiment were obtained by slow evaporation at room temperature in CH_3OH solution. Therefore, the structure and stereochemistry of **1** were assigned by this experiment^[23] (Figure 4). Finally, the final refinement in the $\text{CuK}\alpha$ data resulted in a suitable low Flack parameter 0.1(2), allowing an unambiguous assignment of the absolute configuration of **1** as *2R,7S,9S,15R*, and *8'R*^[24].

Figure 3. Key NOESY correlations of **1** and **2**

Figure 4. X-ray structure of **1**

Norsecurinamine B (**2**) was obtained as colorless needles. The HR-ESI-MS of **2** showed a quasi-molecular ion at m/z 424.2231 [$\text{M} + \text{H}$]⁺ (calcd for $\text{C}_{24}\text{H}_{30}\text{N}_3\text{O}_4$: 424.2231), consistent with the

molecular formula $C_{24}H_{29}N_3O_4$. The 1H NMR spectrum of **2** also showed the typical signals corresponding to an α,β -unsaturated γ -lactone ring [δ_H 5.60 (1H, d, $J = 2.2$ Hz)]. The ^{13}C NMR and DEPT spectra revealed the presence of twelve carbon signals including signals deduced to an α,β -unsaturated γ -lactone ring (δ_C 172.9, 172.3, 110.0, and 91.7). With the aid of 1D and 2D NMR experiments, the 1H and ^{13}C NMR data of **2** were assigned as shown in Table 2. The NMR data of **2** were very similar to those of dihydronorsecurinine, except for the methylene at C-15 of dihydronorsecurinine was replaced by a methine in **2** as well as the significant down field shift of the signal assigned to C-15 (δ_C 56.3). The above NMR spectral data coupled with the molecular formula information suggested that **2** might be a symmetrical norsecurinine-type alkaloid dimer linked via an amino function located at C-15.

In the HMBC spectrum, correlations between H-12 (H-12') and C-9 (C-9')/C-14 (C-14'), between H-2 (H-2') and C-13 (C-13'), between H-5 (H-5') and C-2 (C-2'), as well as between H-8 (H-8') and C-13 (C-13') validated the presence of norsecurinine-type alkaloid skeleton in **2** (Figure 2). The single crystal X-ray diffraction analysis^[25] further confirmed the complete structure of **2** as a dimeric norsecurinine-type alkaloid combined through C-15-NH-C-15' bond. The absolute configuration of **2**^[25] was also well defined with a small Flack parameter of 0.0(2) shown in Figure 5.

Figure 5. X-ray structure of **2**

Table 2. NMR data of **2** (in $CDCl_3$, J in Hz)^a

No.	δ_H	δ_C
2 (2')	3.11	66.8
3 (3')	a 1.88 m b 1.76	28.9
4 (4')	a 1.95 m b 1.75	26.6
5 (5')	a 3.37 m b 2.62 m	58
7 (7')	3.01	64.5
8 (8')	a 2.50 dd (11.3, 6.1) b 1.31 d (11.3)	32.9
9 (9')	-	91.7
11 (11')	-	172.9
12 (12')	5.60 d (2.2)	110
13 (13')	-	172.3
14 (14')	a 3.01 b 2.38 m	31
15 (15')	2.78 m	56.3

^a Overlapped signals were reported without designating multiplicity.

Compound **1** represents the first example of hetero-dimeric alkaloid comprised of norsecurinine and donaxaridine units. Donaxaridine [3-hydroxy-3-(2'-aminophenyl)-*N*-methyl-2-pyrrolidone], belonging to 3-hydroxy-2-oxindole derivatives, is a rare bicyclic molecule consisting of 2-aminophenyl and substituted pyrrolidone rings connected through C-C bond. To date, donaxaridine and its *N*-methyl derivative chimonamidine have only been found in *Arundo donax* (Gramineae) and *Chimonanthus praecox* (Calyanthaceae)^[27,29]. Moreover, compound **1** is also the first structure with donaxaridine unit from family Euphorbiaceae. On the basis of literature and our findings, the biosynthetic pathway of **1** could be proposed to be generated from norsecurinine (**4**) and a key intermediate **A** through Michael addition reaction^[28] as shown in Scheme 1. Intermediate **A** could be yielded from *N*-methyltryptamine (**3**), a co-existence tryptophan derivative^[29,30] isolated from the same plant material (see the Supporting Information), undergoing a series of oxidation, methylation, and cyclization reactions.

Scheme 1. Plausible Biogenetic Route of **1**

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23. $C_{23}H_{29}N_3O_5$, $M = 427.49$, monoclinic, space group $P2_1$; $a = 6.4683$ (3) Å, $b = 8.8763$ (5) Å, $c = 18.4197$ (8) Å, $\alpha = 90^\circ$, $\beta = 92.467$ (4)°, $\gamma = 90^\circ$, $V = 1056.58$ (9) Å³, $T = 173$ (10)K, $Z = 2$, $D_x = 1.344$ g/cm³, $F(000) = 456$, A total of 4249 reflections were collected in the range $4.81 \leq \theta \leq 62.68$, of which 2450 unique reflections with $I > 2\sigma(I)$ were collected for the analysis. The final values were $R = 0.0314$, $R_w = 0.0807$, and the goodness of fit on F^2 was equal to 1.055. Flack parameter = 0.1(2). The crystal data of compound **1** was deposited with the Cambridge Crystallographic Data Centre (CCDC 1480956, <http://www.ccdc.cam.ac.uk/>)
24. Norsecurinamine A (**1**): colorless needles (CH₃OH); mp 201-202°C; $[\alpha]_D^{20} = -20.0^\circ$ ($c = 0.1$, CH₃OH); HR-EI-MS m/z 410.2077 $[M + H]^+$ (calcd for $C_{23}H_{28}N_3O_4$: 410.2074); UV λ_{max} (CH₃OH): 206, 256, 294 nm; IR (KBr) ν_{max} : 3351, 2948, 2893, 1763, 1687, 1455, 1111 cm⁻¹; ¹H NMR (400 MHz, CD₃OD) and ¹³C NMR (100 MHz, CD₃OD) spectroscopic data, see Table 1.
25. $C_{24}H_{29}N_3O_4$, $M = 423.50$, orthorhombic, space group $P2_12_12_1$; $a = 6.8162$ (4) Å, $b = 19.0040$ (13) Å, $c = 22.9252$ (15) Å, $\alpha = \beta = \gamma = 90^\circ$, $V = 2969.6$ (3) Å³, $T = 173$ (2)K, $Z = 4$, $D_x = 0.947$ g/cm³, $F(000) = 904$. A total of 8068 reflections were collected in the range $3.86 \leq \theta \leq 62.61$, of which 3755 unique reflections with $I > 2\sigma(I)$ were collected for the analysis. The final values were $R = 0.0777$, $R_w = 0.2216$, and the goodness of fit on F^2 was equal to 1.059. Flack parameter = 0.0(2). The crystal data of compound **2** was deposited with the Cambridge Crystallographic Data Centre (CCDC 1480957, <http://www.ccdc.cam.ac.uk/>)
26. Norsecurinamine B (**2**): colorless needles (CH₃OH); mp 209-210°C; HR-ESI-MS m/z 424.2231 $[M + H]^+$ (calcd for $C_{24}H_{30}N_3O_4$: 424.2231); UV λ_{max} (CH₃OH): 216 nm; IR (KBr) ν_{max} : 3456, 2961, 1751, 1648, 1581, 1451 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) and ¹³C NMR (75 MHz, CDCl₃) spectroscopic data, see Table 2.
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