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Ningpoensines A–C: unusual zwitterionic alkaloids from *Scrophularia ningpoensis*

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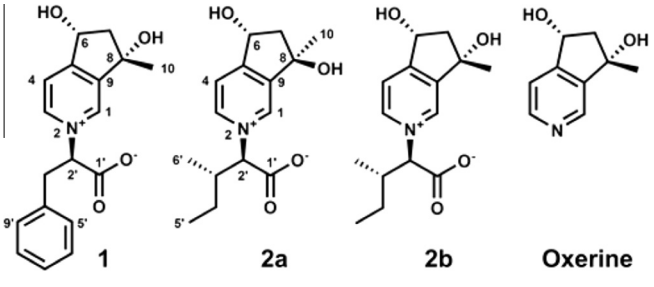
ABSTRACT

Three novel zwitterionic alkaloids—ningpoensine A (**1**) and a pair of epimers, ningpoensines B/C (**2a**/**2b**)—with unprecedented molecular skeletons were isolated from the root of *Scrophularia ningpoensis*. Their structures were established by extensive spectroscopic analyses, and the absolute configuration of ningpoensine A was determined by quantum chemical calculations. A biosynthetic pathway leading to ningpoensines A–C from harpagide and natural amino acids is proposed. Ningpoensines B/C tended to promote wound closure in human embryonic keratinocytes.

Scrophularia ningpoensis (family: *Scrophulariaceae*) is widely distributed in South and Northwest China. Its dried root is prescribed in traditional Chinese medicine (TCM) decoctions for the treatment of fever, swelling, and inflammation.[1] Previous phytochemical investigations show that *S. ningpoensis* is a rich source of sugar esters and iridoid glycosides;[2] many of these metabolites exhibit a wide spectrum of biological effects such as antioxidative, antitumor, and neuroprotective activities.[3]

Wounds often cause major complications underlying several diseases such as diabetes, hematomas, and contusions as well as surgical complications. Therefore, developing an efficient and reliable wound healing-promoting agent is of extreme importance not only for improving patient quality of life but also reducing healthcare costs. TCMs have been used to promote wound healing in China for centuries. For example, Yunnan Baiyao is the most well-known household TCM in China; it is used to stop bleeding and promote the healing of wounds and external injuries.[4] As part of our ongoing efforts to discover novel and bioactive natural products, we have isolated 3 structurally unique zwitterionic alkaloids, ningpoensines A (**1**) and B/C (**2a**/**2b**), from *Scrophularia ningpoensis*. Herein, we report the isolation, structural elucidation, plausible biogenetic pathway, and biological activities of these unusual metabolites.

First, 70% EtOH/H_2O crude extract of the dried root of *S. ningpoensis* was suspended in water and subsequently extracted with petroleum ether, ethylacetate, and *n*-butanol. From the *n*-butanol extract, 3 novel alkaloids, ningpoensines A (**1**) and B/C (**2a**/**2b**), were isolated by serial column chromatography on macroporous resin DA101, silica gel, Sephadex LH-20, and preparative reversed-phase silica high-performance liquid chromatography (HPLC).



Compound **1** was obtained as a brown powder,[5] and the molecular formula was determined to be $C_{18}H_{19}NO_4$ by high-resolution

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electrospray ionization mass spectrometry (HRESIMS; *m*/*z*: 314.1402 [M+H]$^+$, calculated: 314.1392). The ^{1}H nuclear magnetic resonance (NMR) spectrum of **1** (Table 1) indicated the presence of one monosubstituted benzene ring at δ_H 7.05–7.08 (2H, m, H-6′, and H-8′) and 7.16–7.19 (3H, m, H-5′, H-7′, and H-9′), one 3, 4-disubstituted pyridine ring at δ_H 7.86 (1H, d, J = 6.3 Hz, H-4), 8.66 (1H, s, H-1), and 8.76 (1H, d, J = 6.3 Hz, H-3), one oxygenated methine at δ_H 5.16 (1H, dd, J = 8.7, 7.5 Hz, H-6), one singlet methyl at δ_H 1.34 (3H, s, H-10), and one methylene at δ_H 2.76 (1H, dd, J = 12.6, 7.5 Hz, H-7a) and 2.16 (1H, dd, J = 12.6, 8.7 Hz, H-7b). These findings are corroborated by the ^{13}C NMR spectrum (Table 1), suggesting compound **1** contains oxerine, a monoterpene isolated from *Oxera morieri*, as its subunit when compared with previous data.[6] This was confirmed by 2D NMR analyses including ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear single quantum coherence spectroscopy (HSQC), and heteronuclear multiple-bond correlation spectroscopy (HMBC) (Fig. 1). The ^{1}H–^{1}H COSY correlations of H-3/H-4 and H-6/H-7 as well as the HMBC correlations from H-6 to C-5 (δ_C 164.1), H-4 to C-6 (δ_C 71.9), H-7a to C-9 (δ_C 149.5)/C-10 (δ_C 28.8), H-1 to C-3 (δ_C 145.7)/C-5, and H-10 to C-9 (δ_C 149.5)/C-8 (δ_C 77.0) indicate the presence of an oxerine unit in compound **1**. In addition, the presence of a dihydrocinnamate moiety in **1** was revealed through the diagnostic HMBC correlations from H-3′b at δ_H 3.36 (1H, dd, J = 14.7, 12.0 Hz) to C-4′ (δ_C 137.4)/C-5′ (δ_C 130.0) and H-2′ at δ_H 5.46 (1H, dd, J = 12.0, 3.9 Hz) to C-1′ (δ_C 171.2)/C-4′ together with the ^{1}H–^{1}H COSY correlations of H-2′/H-3′. Finally, the planar structure elucidation of compound **1** was completed by connecting the oxerine and dihydrocinnamate moieties via an N–C bond between N-2 and C-2′, which was justified by the key HMBC correlations from H-2′ to C-1 (δ_C 141.7) and H-1/H-3 to C-2′ (δ_C 78.8). The detailed analyses of the molecular formula and unsaturation degree of **1** combined with the infrared (IR) absorption (1629 cm^{-1}) characteristics in the presence of carboxylate ion[7] support the zwitterionic structure of compound **1**. The relative configurations of C-6 and C-8 were elucidated by nuclear Overhauser effect spectroscopy (NOESY) (Fig. 1). The NOE correlation of H-6 with CH_3-10 indicated the hydroxyl groups at both C-6 and C-8 were located on the same face. Therefore, compound **1** was determined to be an unusual zwitterionic alkaloid and named 'ningpoensine A'.

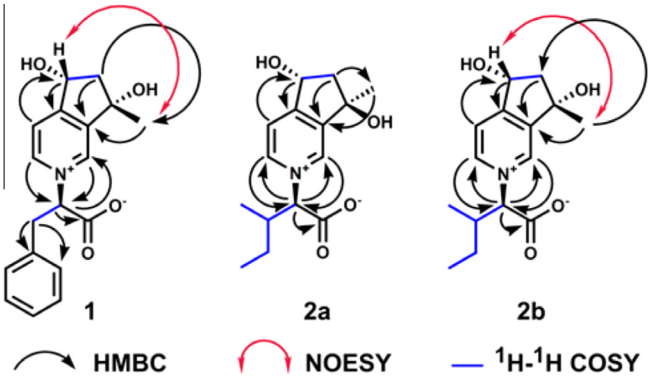

Figure 1. Key ^{1}H–^{1}H COSY, HMBC, and NOESY correlations of compounds **1** and **2a**/**2b**.

To determine the absolute configuration of **1**, varieties of crystallization methods have been explored aiming to obtain single-crystals. However, no desired crystal suitable for X-ray diffraction analysis was obtained. Pleasingly, the method of quantum chemical calculations of electronic circular dichroism (ECD) spectra has been proven to be very accurate and reliable for deducing the absolute configurations of natural products.[8] The predicated ECD spectra of 4 possible isomers of **1**-2′*R*/6*R*/8*S*, 2′*S*/6*R*/8*S*, 2′*R*/6*S*/8*R*, and 2′*S*/6*S*/8*R*—were calculated using the TD-DFT theory method at the B3LYP/6-311+G(d,p) level (Supporting information). By comparing the experimental CD spectrum with the theoretically calculated CD curves (Fig. 2), the absolute configuration of compound **1** was tentatively assigned as 2′*R*/6*R*/8*S*.

Compounds **2a**/**2b** were also obtained as a brown powder;[9] their molecular formulas were both determined to be $C_{15}H_{21}NO_4$ by HRESIMS (*m*/*z*: 280.1561 [M+H]$^+$, calculated: 280.1549). Similar to **1**, the IR spectra of **2a**/**2b** showed strong absorption bands for both the hydroxyl group (3368 cm^{-1}) and carboxylate ion (1625 cm^{-1}), indicating the zwitterionic structures of **2a**/**2b**. The ^{1}H and ^{13}C NMR spectra (Figs. S9, S10, Supporting information) of compounds **2a**/**2b** showed 2 sets of signals in a ratio of 2:1,

Table 1
^{1}H (300 MHz) and ^{13}C NMR (75 MHz) data of compounds **1** and **2a**/**2b** in CD_3OD

Pos	1		2a		2b	
	δ_H (mult, J in Hz)	δ_C	δ_H (mult, J in Hz)	δ_C	δ_H (mult, J in Hz)	δ_C
1	8.66 (s)	141.7	9.02 (s)	141.2	9.02 (s)	141.2
2	—	—	—	—	—	—
3	8.76 (d, 6.3)	145.7	9.03 (d, 6.3)	145.8	8.94 (d, 6.3)	145.8
4	7.86 (d, 6.3)	123.7	8.03 (d, 6.3)	124.2	7.99 (d, 6.3)	123.8
5	—	164.1		166.2		164.3
6	5.16 (dd, 8.7, 7.5)	71.9	5.51 (dd, 7.2, 6.9)	73.3	5.26 (dd, 8.7, 7.5)	72.0
7	(a) 2.76 (dd, 12.6, 7.5) (b) 2.16 (dd, 12.6, 8.7)	54.0	(a) 2.70 (dd, 13.5, 6.9) (b) 2.13 (dd, 13.5, 7.2)	52.4	(a) 2.83 (dd, 12.6, 7.5) (b) 2.22 (dd, 12.6, 8.7)	54.1
8	—	77.0	—	78.2	—	77.1
9	—	149.5	—	148.1	—	149.6
10	1.34 (s)	28.8	1.75 (s)	27.3	1.54 (s)	28.7
1′	—	171.2	—	171.4	—	171.4
2′	5.46 (dd, 12.0, 3.9)	78.8	4.83[a]	83.0	4.83[a]	83.0
3′	(a) 3.89 (dd, 14.7, 3.9) (b) 3.36 (dd, 14.7, 12.0)	41.0	2.43 (m)	39.6	2.43 (m)	39.6
4′	—	137.4	(a) 1.04 (m) (b) 1.22 (m)	26.2	(a) 1.04 (m) (b) 1.22 (m)	26.2
5′	7.16–7.19 (m)	130.0	0.89 (t, 7.2)	11.2	0.89 (t, 7.2)	11.2
6′	7.05–7.08 (m)	129.7	1.13 (d, 6.6)	16.2	1.13 (d, 6.6)	16.2
7′	7.16–7.19 (m)	128.3				
8′	7.05–7.08 (m)	129.7				
9′	7.16–7.19 (m)	130.0				

[a] Masked by solvent peak.