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Sinoscrewtine, an alkaloid with novel skeleton from the roots of *Sinomenium acutum*

Xiao-Ling Wang [a,*], Bing-Rui Liu [b], Chien-Kuang Chen [d], Guo-Wei Qin [c], Shoei-Sheng Lee [d]

[a] Key Laboratory of Phytochemistry of Shaanxi Province, Baoji University of Arts and Sciences, Baoji 721013, China
[b] College of Science, Northwest Agriculture and Forest University, Yangling 712100, China
[c] Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 201203, China
[d] School of Pharmacy, College of Medicine, National Taiwan University, Taipei 100, China

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ABSTRACT

An alkaloid with novel skeleton, sinoscrewtine (**1**), has been isolated from the roots of *Sinomenium acutum*. Its structure was established by spectral analysis and X-ray crystallographic study, and its possible biosynthetic pathway was delivered. In vitro experiments, **1** showed weak injurious effects against $H_2O_2/A\beta_{25-35}$ induced oxidative injury in PC-12 cells and DPPH radical scavenging activity with IC_{50} of 32.6 μM.

1. Introduction

Sinomenium acutum (Thunb.) Rehd. et Wils. is a Menispermaceaeous medicinal plant widely distributed in China [1]. The stems of this plant have been recorded in the Chinese Pharmacopeia as a traditional herbal medicine for the treatment of rheumatalgia, rheumatism, and arthralgia [2]. It is known that *S. acutum* contains abundant alkaloids with diversified skeletons [3–9]. Sinomenine, a known morphinan-type alkaloid, is the main component of the plant and showed inhibitory effect for inflammatory reaction and lymphocyte proliferation, and so has being used as an anti-arthritic drug clinically [10]. In our previous study, two new morphinan-type alkaloids [11], two new morphinan-type alkaloid dimmers [12], a new hausbanan-type alkaloid [13], and an alkaloid with new skeleton in which sinoacutine was considered as its bioprecursor [14] had been reported from the stems or roots of this plant. In order to find more active components, the residue after the removal of sinomenine was investigated and resulted in the isolation of a new skeleton alkaloid named sinoscrewtine (**1**) which its bioprecursor was considered to be sinomenine (Fig. 1). Here we report the isolation, structural determination, the possible biosynthetic path, and the effects against $H_2O_2/A\beta_{25-35}$ induced oxidative injury in PC-12 cells and DPPH radical scavenging activity of **1**.

2. Experimental

2.1. General

IR spectrum (KBr) was obtained on a JASCO FT/IR-410 spectrometer. UV spectrum was recorded on a Hitachi U-2001 spectrophotometer. HR-ESI-MS was obtained on a Finnigan MAT TSQ 7000 spectrometer. ^1H, ^{13}C and 2D NMR data were determined on a Bruker AVANCE 600 instrument in $CDCl_3$ and the chemical shifts were referenced to the residual solvent peak of $CDCl_3$. All solvents used were of analytical grade. Silica gel (200–300 mesh and 300–400 mesh) was used for column chromatography, and precoated silica GF254 plates were used for TLC (Qingdao Haiyang Chemical Company, Ltd.). Activity tests were determined on a Bio Tek Powerwave

* Corresponding author. Tel.: +86 13892743101.
E-mail address: xlwangwang@163.com (X.-L. Wang).

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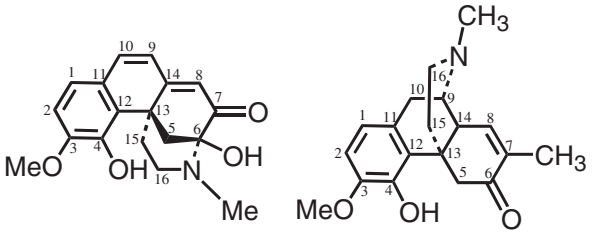

Fig. 1. The structure of sinoscrewtine (1) and sinomenine.

XS2 automated microplate. X-ray diffraction was performed on a Bruker SMART APEX-II diffractometer equipped with a graphite-monochromatic Cu*Kα* radiation (λ = 1.54178 Å).

2.2. Plant materials

The roots of *S. acutum* were collected in Qinling Mountains, Shaanxi Province, China, and identified by Dr. Xiaomei Wang of Baoji University of Arts and Sciences. A voucher specimen was deposited in herbarium in the Key Laboratory of Phytochemistry of Shaanxi Province, Baoji University of Arts and Sciences.

2.3. Extraction and isolation

The powder of the roots of plant were soaked in 10% $Ca(OH)_2$ solution, and then extracted with benzene. The benzene extract was concentrated and stand for overnight to precipitate the major alkaloid sinomenine. After removal of the crude sinomenine, the mother liquor was concentrated to be a sticky residue.

The sticky residue (3 kg) was fractionated by column chromatography on silica gel (100 mesh) eluted with $CHCl_3$ to $CHCl_3$/MeOH (30:1; 10:1 and 4:1), gradually to afford 7 fractions, QT1–QT7. The QT3 (600 g) was subjected to column chromatography on silica gel (200–300 mesh) eluted with petroleum ether/acetone (10:1 to 100% acetone) to give 12 subfractions, QT3-1–QT3-12. The QT3-11 was chromatographed repeatedly on silica gel (300–400 mesh) eluted with $CHCl_3$/MeOH (100:1 to 20:1) to yield **1** (22 mg).

Sinoscrewtine (**1**), slight orange crystals, $[\alpha]_D^{25}$-258.2 (c 0.402 in MeOH), HR-ESI-MS: *m/z* 314.1387 $[M+H]^+$, 336.1206 $[M+Na]^+$ (calcd. 314.1392 for $C_{18}H_{20}NO_4$ and 336.1212 for $C_{18}H_{19}NNaO_4$, respectively). UV(MeOH) λ_{max} are 391 (0.459), 254 (0.436) and 224 (0.711) nm. IR (KBr cm^{-1}) are 3510, 3467, 2935, 2850, 1643, 1609, 1561, 1467, and 1436. ^1H NMR and ^{13}C NMR, see Table 1.

X-ray crystallographic study was performed with a colorless single crystal (dimensions of 0.16 mm × 0.14 mm × 0.11 mm obtained from ethyl acetate) on a Bruker SMART APEX-II diffractometer equipped with a graphite-monochromatic Cu*Kα* radiation (λ = 1.54178 Å) using f and ω scans at 289 (2) K. The diffraction revealed that **1** crystallized as $C_{18}H_{19}NO_4$, in monoclinic system, space group $P2_1$, with a = 6.9913 (10) Å, b = 7.8945 (10) Å, c = 13.5762(10) Å, α = 90°, β = 90.14°, γ = 90°, Z = 2, D_x = 1.389 g/cm^3, $F(000)$ = 332, μ(Cu*Kα*) = 0.81 mm^{-1}, the final R = 0.0301 and wR = 0.0823 for 2101 independent reflections with R_{int} = 0.0136 and 2088 observed reflections with $I > 2\sigma(I)$. See Supplementary data for ORTEP view for **1**.

Table 1
^1H, ^{13}C and ^2D NMR data of sinoscrewtine (1).

Position	δ_H (mult, *J*, Hz) [a,b]	δ_C (mult) [a,c]	HMBC (HC)	NOESY(HH)
1	6.77(d, *J* = 8.2)	121.27(d)	C-3, C-10, C-12	H-2
2	6.79(d, *J* = 8.2)	108.71(d)	C-4, C-11	H-1, 5-OCH_3
3		148.08(s)		
4		144.41(s)		
5	2.23(d, *J* = 15.6, H-5a) [d]			H-5e, H-15a, H-16a, H-16e
	3.92(d, *J* = 15.6, H-5e) [e]	43.49(t)	C-7, C-12, C-14, C-15	H-5a
6		83.20(s)		
7		194.08(s)		
8	6.13(s)	124.08(d)	C-6, C-9, C-13	H-9
9	6.30(d, *J* = 9.4)	123.40(d)	C-8, C-11, C-13	H-10, H-8
10	6.70(d, *J* = 9.4)	135.97(d)	C-1, C-12, C-14	H-9
11		125.61(s)		
12		125.48(s)		
13		44.36(s)		
14		161.43(s)		
15	2.71, td(*J* = 13.1, 4.9, H-15e)			H-15a, H-16e
	1.38, brd(*J* = 13.1, H-15a)	31.22(t)	C-5, C-12, C-14	H-5a, H-16a, H-16e
16	2.89, dd(*J* = 12.1, 4.9, H-16e)	46.63(t)	C-6, C-13	H-16a, H-15e, NCH_3
	2.59, td(*J* = 12.1, 3.4, H-16a)			H-5a
5-OCH_3	3.94(s)	56.19(q)	C-3	H-2
NCH_3	2.25(s)	36.21(q)	C-6, C-16	H-16e

[a] Chemical shifts are in ppm in $CDCl_3$.
[b] 600 MHz in $CDCl_3$.
[c] 150 MHz in $CDCl_3$.
[d] Proton of axial bond.
[e] Proton of equatorial bond.

3. Results and discussion

Chromatographic separation on the residue after the removal of sinomenine from the roots of *S. acutum* led to the isolation of the title compound, sinoscrewtine (**1**). Sinoscrewtine was obtained as slight orange crystals. Its quasi molecular ions $[M+H]^+$ at *m/z* 314.1387 and $[M+Na]^+$ at m/z 336.1206 obtained from HR-ESI-MS indicated the molecular formula of $C_{18}H_{19}NO_4$ (calcd. 314.1392 and 336.1212, respectively). The UV absorptions at λ_{max} 224 (0.711), 254 (0.436), and 391 (0.459) nm revealed that **1** is highly conjugated. The IR spectrum showed absorption bands for hydroxyl (3510, 3467 cm^{-1}), conjugated carbonyl (1643 cm^{-1}), and aryl (1609 cm^{-1}) group. ^{13}C NMR and DEPT gave 18 signals for 2 methyl (one oxygenated and another aminated), 3 methylene (all saturated), 5 methine (all aromatic and/or olefinic), and 8 quaternary carbons (one carbonyl, five aromatic and/or olefinic, and two saturated). ^1H NMR revealed the presence of one pair of aromatic ortho-position protons (δ_H 6.77 ppm, 1H, d, *J* = 8.2 Hz, H-1; δ_H 6.79 ppm, 1H, d, *J* = 8.2 Hz, H-2), one pair of olefinic protons of cis-position (δ_H 6.30 ppm,