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Sinoracutine, a novel skeletal alkaloid with cell-protective effects from *Sinomenium acutum*

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ABSTRACT

From the stems of *Sinomenium acutum*, sinoracutine (**1**) has been isolated, an alkaloid with a structurally novel skeletal framework, whose structure has been established by spectral and single crystal X-ray diffraction analysis. In vitro experiments show that sinoracutine increases cell viability against Hydrogen peroxide-induced oxidative injury.

The plant family Menispermaceae is well known for the production of a wide variety of alkaloids. Of the three reviews on alkaloids isolated from this family, recent one published in 2000 alone described 1525 alkaloid constituents, encompassing 22 different structural types.[1] The benzyltetrahydroisoquinoline derived from the shikimate biosynthetic pathway has been recognized as an important intermediate in the formation of almost all the alkaloids in the plants of this family.

The species *Sinomenium acutum* (Thunb.) Rehd. Et Wils. Of the Menispermaceae family is a deciduous twining vine distributed widely in China. The stems of this plant have been identified in the Chinese Pharmacopeia (2005 edition) as a traditional medicine for the treatment of rheumatagia, rheumatism, and arthralgia.[2] Previous phytochemical studies of this plant have led to the isolation of a number of alkaloids, including the morphinan sinomenine (**2**), which inhibits inflammatory reactions and lymphocyte proliferation, and is used clinically as an anti-arthritic drug.[3] In the course of our work on sinomenine, from the stems of the same plant, we have isolated a new alkaloid sinoracutine (**1**), which has an unprecedented skeletal structure and has demonstrated cell-protective activity. In this Letter, we report the isolation and structural determination of **1**, together with studies on its protection of cells against free hydrogen peroxide-induced injury.

Chromatographic separation on the ethanol extracts of the stem of the plant *Sinomenium acutum* led to the isolation of a new skeletal alkaloid, sinoracutine (**1**).[4] Sinoracutine was obtained as colorless crystals and its molecular ion $[M]^+$ at *m/z* 283.1207 obtained from HREIMS indicates a molecular formula of $C_{17}H_{17}NO_3$ (calcd 283.1209). The UV absorptions recorded at λ_{max} 223 (0.377), 251 (0.233), and 393 (0.160) nm revealed that **1** is highly conjugated. The IR spectrum of **1** showed absorption bands for hydroxyl (3438, 3062 cm^{-1}), conjugated carbonyl (1673 cm^{-1}), and aryl (1600 cm^{-1}) groups. The ^{13}C NMR spectrum of **1** of 17 signals consisted of two methyl (one oxygenated and one aminated), two methylene, six methine (two aromatic, three olefinic, and one saturated), and seven quaternary (one carbonyl, four aromatic, one saturated, and one olefinic) carbon atoms. The ^1H–^1H COSY spectrum revealed the presence of an isolated $-CH_2CH_2-$ fragment and two pairs of vicinal protons (Fig. 2). The HMQC spectrum established all one-bond ^1H–^{13}C connectivities (Table 1). Further examination of the ^1H, ^{13}C, and 2D NMR data, together with consideration of its degree of molecular unsaturation, suggested that compound **1** possesses a 6/6/5/5 tetracyclic skeleton bearing *N*-methyl, methoxy, hydroxyl, and cyclopentenone moieties, which differ significantly from the ring system of sinomenine, the morphinan alkaloid previously isolated as a major constituent of this plant. The HMBC spectrum revealed significant correlations (Table 1, Fig. 2) between H-1 and C-3, C-10, C-12; between H-2 and C-4, C-11; H-5 and C-7, C-12, C-15, C-16; between H-9 and C-13, and H-10 and C-1, C-12, C-14, which supported the proposed structure of **1**. Finally, the structure and relative configuration of **1** were unambiguously confirmed by X-ray crystallographic analysis (Fig. 3).[5]

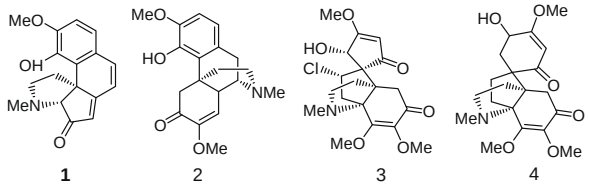

Figure 1. Sinoracutine **1**, and related menispermaceae alkaloids

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Table 1
^{1}H and ^{13}C NMR data for sinoracutine **1**

Position	δ_H (mult, *J*, Hz)[a,b]	δ_C (mult)[a,c]	HMBC (HC)
1	6.75 (d, 8.2)	121.24 (d)	C-3, 10, 12
2	6.73 (d, 8.2)	109.42 (d)	C-4, 11
3		151.07 (s)	
4		145.58 (s)	
5	3.77 (s)	72.31 (d)	C-7, 12, 15, 16
7		206.56 (s)	
8	5.84 (s)	123.27 (d)	C-5, 7, 9
9	6.54 (d, 9.4)	118.00 (d)	C-11, 13
10	6.78 (d, 9.4)	138.10 (d)	C-1, 12, 14
11		124.11 (s)	
12		127.59 (s)	
13		53.71 (s)	
14		174.94 (s)	
15	2.73 (m)	41.52 (t)	
	2.10 (m)		
16	3.18 (dd, 9.7, 8.7)	51.46 (t)	C-5, 13, 15
OCH_3	3.91 (s)	55.87 (q)	C-3
NCH_3	2.92 (s)	36.35 (q)	

[a] Chemical shifts are in ppm downfield of internal TMS in $CDCl_3$.
[b] 500 MHz in $CDCl_3$.
[c] 125 MHz in $CDCl_3$.

Literature investigations revealed that the structural skeleton of **1** has never been reported. As mentioned previously, the family Menispermaceae produces a rich diversity of alkaloid metabolites. Compared with compounds isolated from the same plant, **1** is related to the morphinan sinomenine **2**, but the piperidine ring has been fused onto the framework as a five-membered pyrrolidine. Acutumine **3** and acutudaurin **4** (Fig. 1), spirocyclic alkaloids also isolated from plants of the same family, also feature this fused pyrrolidine structure.[6,7] Moreover, the cyclohexenone also underwent contraction to yield a cyclopentenone as the final ring. Previous biosynthetic studies firmly established (*S*)-reticuline as the precursor of morphinandienone alkaloids, sinoacutine, involving intramolecular oxidative coupling of phenoxy-radicals.[8–10] We propose a possible biosynthetic pathway for **1** as shown in Scheme 1, in which the biogenetic precursors reticuline and sinoacutine have also been isolated from the same plant in our investigations.[11,12] Sinoacutine is proposed to undergo a nitrogen-related oxidation, reduction, hydrolysis, and elimination, followed by Micheal addition of the alkylamine to the less sterically hindered enone to construct pyrrolidine **5**. A similar conjugate addition reaction has been appeared recently in a synthetic effort toward the related alkaloid acutumine **3**.[13] Subsequent Baeyer–Villiger oxidation[14] followed by hydrolysis, Dieckmann-type condensation, and decarboxylation, would result in a ring-contracted cyclopentenone. Based on this biosynthetic pathway, from sinoacutine, the configuration of the benzyl quaternary carbon C-13 being kept (*R*) and C-13 attending the formation of this five-membered pyrrolidine ring together with C-5, the pyrrolidine ring should fuse the cyclopentenone at C5–C13 from the back and the configuration of the tertiary carbon C-5 should be deduced as (*R*). This deduction was further conformed by the minus optical rotation value ($[\alpha]_D^{25}$ −7.4 (c 0.35 in $CHCl_3$), and its CD spectrum (220 nm, +13; 270 nm, −1) (see the Supplementary data).

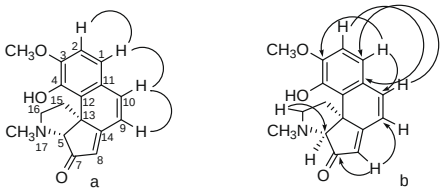


Figure 2. ^{1}H–^{1}H COSY (a) and key HMBC correlations (b) of **1**.

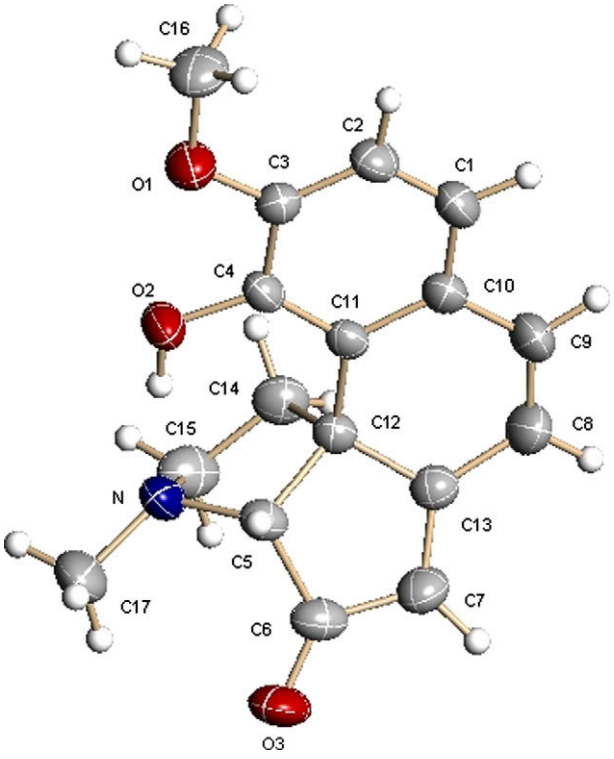
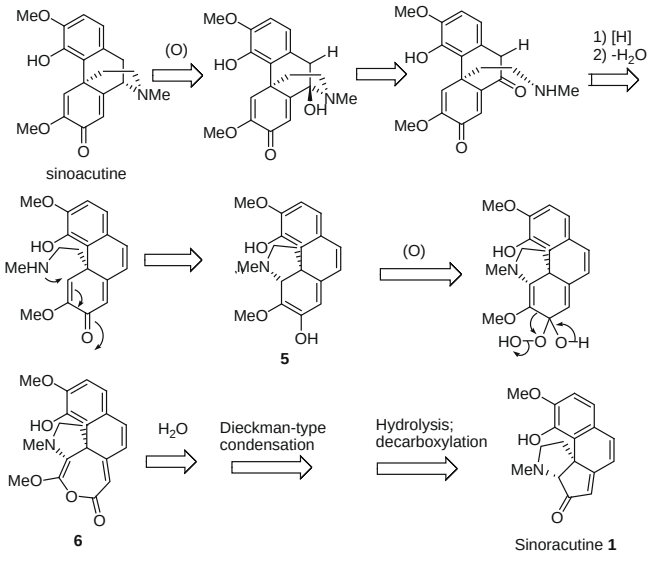


Figure 3. Perspective view of **1**.

Various neurodegenerative disorders, including Alzheimer's disease (AD), attribute neuronal degeneration and death to oxidative stress by reactive oxygen species (ROS).[15,16] Hydrogen peroxide (H_2O_2) is an ROS and a readily diffusable precursor for the highly active hydroxyl radical. The characteristic pathological features of neuronal cell death and loss of synaptic function could be induced by elevated levels of H_2O_2. Moreover, the neurotoxic β-amyloid (Aβ) peptide that deposits as characteristic fibril plaques in AD pathology generates H_2O_2;[17] and its toxicity has also been found to be mediated by H_2O_2.[18,19] Thus some antioxidants have been effective in protecting cells from Aβ toxicity, and therapeutic efforts aimed at removal of free radicals or prevention of their formation may constitute a treatment for AD.[20–22] The cell-protective effect of **1** against oxidative stress was probed by studies on



Scheme 1. A proposed biosynthesis of sinoracutine **1**.

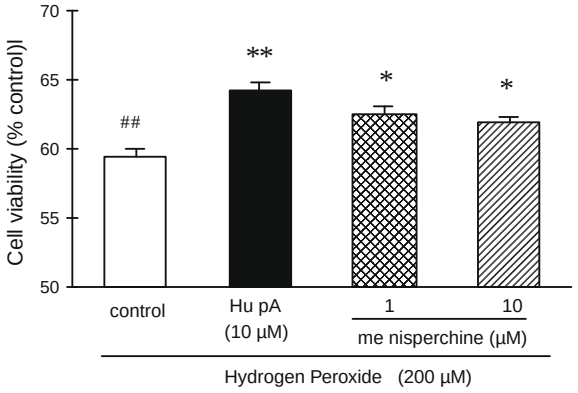


Figure 4. Effect of sinoracutine **1** and huperzine A on cell viability, induced by H_2O_2 damage in PC12 cells. Cell viability was assessed by measuring MTT reduction after 24 h.[24] Shown are mean ± SD expressed as percentage of control. One-way ANOVA followed by Duncan's test was used to test the statistical significance. $^{\#\#}p < 0.01$ versus control, $^{**}p < 0.01$ and $^{*}p < 0.05$ versus H_2O_2 group.

hydrogen peroxide-induced cell lesion in the rat pheochromocytoma line PC12, a useful model of nerve growth factor responsive peripheral neurons.[23] Following a 6-h exposure to H_2O_2 (200 μM), a substantial percentage of the cell population was lysed. Using MTT as a reagent to quantify cell viability,[24] the living cell count was found to have been reduced to 59.4% after H_2O_2 treatment. The pre-incubation of cells with **1** (at 1 and 10 μM) for 2 h prior to H_2O_2 exposure was found to increase cell survival to 62.5% and 61.9%, respectively, therefore demonstrating a significant protective effect against hydrogen peroxide-induced cell toxicity (Fig. 4). These observations were compared with and found to parallel the effects of incubation with huperzine A (10 μM), a known reversible and selective acetylcholinesterase inhibitor with demonstrated efficacy in improving memory deficiency in animal tests.[21,25] Thus sinoracutine **1** could be a new lead as an antioxidant for protection of neurons against oxidative damage and may find applications in AD research and therapy.

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Supplementary data

Supplementary data (extraction and purification procedures, ^1H and ^{13}C NMR spectra, crystallographic data, the ORTEP view of sinoracutine **1** and CD spectrum of **1** and related morphinan alkaloids) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.05.040.

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5. X-ray crystal analysis was performed with a colorless crystal (dimensions 0.556 × 0.477 × 0.069 nm) obtained from $CHCl_3$–MeOH. $C_{17}H_{17}NO_3$, monoclinic, $P2_1$, $a = 8.6507$ (14) Å, $b = 10.4644$ (16) Å, $c = 16.078$ (3) Å, $\alpha = 90°$, $\beta = 104.905(3)$ °, $\gamma = 90$ °, $Z = 4$, $R = 0.0423$, $Rw = 0.0674$ for 1680 independent reflections. See Supplementary data for ORTEP view for **1**.
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