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A novel alkaloid from the fruiting bodies of *Ganoderma sinense* Zhao, Xu *et* Zhang

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Abstract

A new alkaloid, sinensine (**1**), had been isolated from the fruiting bodies of *Ganoderma sinense* Zhao, Xu *et* Zhang. Its structure was elucidated on the basis of 1D and 2D spectral analysis. This alkaloid exhibited activity in protecting the injury induced by hydrogen peroxide oxidation on HUVEC, with EC_{50} value 6.2 μmol/L.

Keywords: Alkaloid; *Ganoderma sinense*; Sinensine

Ganoderma sinense is a traditional Chinese medicine, formerly we isolated several steroids from the fruiting bodies of *G. sinense* [1]. In this paper, a new alkaloid was isolated from the EtOH extracts of this fungus, and its structure was characterized by detailed spectroscopic analysis.

The fruiting bodies of *G. sinense* were purchased in Fujian, and identified by Shuning Yu in Guangdong Detection Center of Microbiology.

The fruiting bodies (10 kg) were extracted with 95% ethanol and filtered, concentrated *in vacuo* and partitioned with petroleum, chloroform and ethyl acetate, respectively. The EtOAc extracts (25 g) were subjected to column chromatography on silica gel, eluted with petroleum–acetone (from 5:1 to 1:1). The fractions 1–6 were combined by monitoring with TLC. Fr. 3 was chromatographed repeatedly on silica gel eluted with $CHCl_3$–MeOH (15:1) or $CHCl_3$–acetone (6:1) to afford compound **1** (6 mg).

Sinensine (**1**), a yellow needle from acetone, with mp >300 °C and $[\alpha]_D^{20}$ +8.7 (c 0.1, MeOH), showed IR absorption bands at 3245 cm^{-1} for hydroxyl groups and 1590 and 1485 cm^{-1} for aromatic rings. Its maximum UV absorption at 263 and 343 nm (MeOH) were also due to the aromatic rings. The EI-MS spectrum showed the $[M]^+$ ion peak at *m/z* 257 (14%), and the fragment ion peaks $[M-OH]^-$ at *m/z* 239 (83%), $[M-C_6H_4O_2]^-$ at *m/z* 149 (19%). The molecular formula $C_{15}H_{15}NO_3$, with nine degrees of unsaturation, was established by the HR-EI-MS which gave the $[M]^+$ ion peak at *m/z* 257.1060 (calcd.257.1052). The ^1H NMR spectrum of **1** (600 MHz, C_5D_5N) exhibited signals to a 1,3,5-trisubstituted phenyl group at δ 8.82 (d, 1H, *J* = 2.4 Hz), 7.31 (d, 2H, *J* = 2.4 Hz), a tetrasubstituted pyridyl group at δ

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Table 1
NMR data of sinensine (**1**) in C_5D_5N (600 MHz for ^1H, 150 MHz for ^{13}C).

No.	δ_C	δ_H	COSY	HMBC
1	153.6 (s)			
3	145.9 (d)	8.10 (s, 1H)	H-3/CH_3-10	H-3/C-1, 4, 9, 10
4	128.7 (s)			
5	29.3 (t)	β 3.04 (m, 1H)	H-5β/H-5α, 6α, 6β	H-5β/C-4, 6, 8, 9
		α 2.59 (dd, 1H, J = 16.8, 8.4 Hz)	H-5α/H-5β, 6α, 6β	H-5α/C-4, 6, 7, 8, 9
6	35.8 (t)	β 2.29 (dd, 1H, J = 13.2, 7.6 Hz)	H-6β/H-5α, 5β, 6α	H-6β/C-5, 7, 8, 9
		α 2.12–2.00 (m, 1H)	H-6α/H-5α, 5β, 6β, 7	H-6α/C-5
7	74.7 (d)	5.57 (d, 1H, J = 6.0 Hz)	H-7/H-6α	H-7/C-5, 8, 9
8	138.8 (s)			
9	156.3 (s)			
10	15.6 (q)	1.98 (s, 3H)	CH_3-10/H-3	CH_3-10/C-3, 4, 9
1′	122.3 (s)			
2′	119.1 (d)	7.31 (d, 1H, J = 2.4 Hz)	H-2′/H-4′, 6′	H-2′/C-3′, 4′
3′	152.8 (s)			
4′	117.8 (d)	8.82 (d, 1H, J = 2.4 Hz)	H-4′/H-2′, 6′	H-4′/C-2′, 3′, 5′, 6′
5′	151.2 (s)			
6′	118.6 (d)	7.31 (d, 1H, J = 2.4 Hz)	H-6′/H-2′, 4′	H-6′/C-1′, 4′, 5′
OH		13.66 (br s, 1H)		
OH		11.04 (br s, 1H)		
OH		4.93 (br s, 1H)		

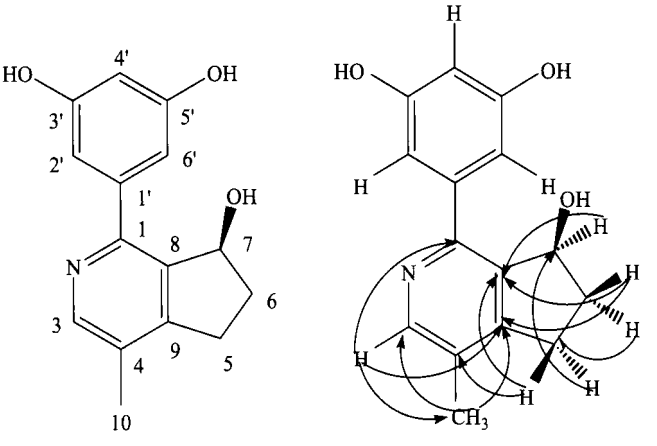

Fig. 1. The structure and key HMBC correlations of sinensine (**1**).

8.10 (s, 1H), a methyl group at δ 1.98 (s, 3H), a methylidyne attached to oxygen at δ 5.57 (d, 1H, J = 6.0 Hz) and three hydroxyl protons at δ 13.66 (br s), 11.04 (br s) and 4.93 (br s). Moreover, the ^{13}C NMR spectrum (Table 1) showed 15 carbon signals, including the signals due to a phenyl group at δ 152.8, 151.2, 122.3, 119.1, 118.6 and 117.8, a pyridyl group at δ 156.3, 153.6, 145.9, 138.8 and 128.7, a methyl group at δ 15.6, a methylidyne attached to oxygen at δ 74.7 and two methylene groups at δ 35.8, 29.3. The final structure of **1** was established by 2D NMR experiments: HSQC and HMBC (Fig. 1).

In the HMBC spectrum, long-range correlations from H-4′ (δ 8.82) to C-2′, C-3′, C-5′and C-6′ (δ 119.1, 152.8, 151.2 and 118.6), H-2′ (δ 7.31–7.30) to C-3′ and C-4′ (δ 152.8 and 117.8), H-6′ (δ 7.31) to C-1′, C-4′ and C-5′ (δ 122.3, 117.8 and 151.2) demonstrated that **1** contained a 1,3,5-trisubstituted phenyl group, and two hydroxyl groups (δ 13.66 and 11.04) were attached to C-3′and C-5′. Meanwhile, long-range HMBC correlations from H-3 (δ 8.10) to C-1, C-4, C-9 and CH_3-10 (δ 153.6, 128.7, 156.3 and 15.6), CH_3-10 (δ 1.98) to C-3, C-4 and C-9 (δ 145.9, 128.7 and 156.3) revealed the presence of the pyridyl group, and the methyl group must be attached to C-4. In addition, HMBC correlations from H-7 (δ 5.57) to C-5, C-8 and C-9 (δ 29.3, 138.8 and 156.3), and also to H6α (δ 2.12–2.00) in the

^1H–^1H COSY spectrum, suggested the existence of a cyclopenteneyl group, and the hydroxyl group (δ 4.93) should be attached to C-7. The configuration of C-7 was confirmed as S according to literature and the NOESY spectrum [2,3].

It was found that compound **1** possessed activity in protecting the injury induced by hydrogen peroxide oxidation on human umbilical cord endothelial cells (HUVEC), with the protective rate 70.90% and EC_{50} value 6.23 μmol/L.

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