SHORT REPORT

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Rec. Nat. Prod. X:X (202X) XX-XX

records of natural products

A New Dihydrophenanthrene with Cell Viability Enhancing Activities from *Spiranthes sinensis*

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(Received October 16, 2024; Revised December 22, 2024; Accepted January 03, 2025)

Abstract: A new dihydrophenanthrene (Spiranthol B), along with four known compounds, were isolated from *Spiranthes sinensis*. The new structure was determined by various spectroscopic analyses such as 1D, 2D NMR and HRMS techniques. Its stereochemistry was resolved by using the calculated electronic circular dichroism (ECD). Compounds **1**-5 showed their capabilities to attenuate palmitic acid (PA)-induced reductions in MIN6 cells viability with the dosages of 3.125 and 6.25 μM.

Keywords: *Spiranthes sinensis*; dihydrophenanthrenes; cell viability.

1. Plant Source

The plant materials were collected from a local market in Bijie City, Guizhou Province, China, in 2021. It was authenticated by Prof. Yuguang Fan from Hainan Medical University, and a voucher specimen was deposited in the Natural Medicinal Chemistry Laboratory of Hainan Medical University with a reference number of FHMU9188.

2. Previous Studies

Spiranthes sinensis, known in Chinese traditional medicine as Panlongshen, is a perennial herb of the *Orchidaceae* family, widely used for its therapeutic properties. This plant is distributed in various Chinese regions, thriving under mountain forests at altitudes between 200 and 3400 meters [1]. It is reputed for the therapeutic functions including nourishing yin and qi, detoxifying, and supporting lung health [2]. Pharmacological studies verified its effects on managing diabetes [1], anti-inflammatory [3], anti-tumor [4-6], and inhibiting adipogenesis [3].

Prior studies have identified a range of chemical constituents in *Spiranthes sinensis*, including dihydrophenanthrenes [6, 7], sterols [7], flavonoids [8], and coumarins [9]. This study adds a newly isolated dihydrophenanthrene to the catalog of bioactive compounds from this plant, highlighting its ability to mitigate PA-induced reductions in MIN6 cells viability.

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The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products Month-Month 202x EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.492.2410.3345
Available online: February 05, 2025

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3. Present Study

Dried whole plant (20.0 kg) of *Spiranthes sinensis* was crushed and extracted by refluxing with 95% ethanol twice for 2 hours each time. A total extract was obtained after filtration and concentration. The extract was then dissolved with water, the mixed solvent was extracted with petroleum ether by three times and concentrated following to give an extract (169.4 g). The petroleum ether extract was further separated by silica gel column chromatography using a petroleum ether-ethyl acetate solvent system, to give fourteen fractions: A-N. Fraction E was separated on Sephadex LH-20 column chromatography using methanol as the eluent to afford six components of E6-1 to E6-6. The E6-1 fraction was purified by HPLC (MeOH: H_2O = 85:15-75:25, v/v) at wavelengths of 210 and 254 nm, with a volume flow rate of 2 mL·min^{-1}, to give compounds **1** (3.0 mg), 2 (6.4 mg). The E6-3 fraction was purified by HPLC (MeOH: H_2O = 80:20, v/v) at wavelengths of 210 and 254 nm, with a volume flow rate of 2 mL·min^{-1}, to afford compounds 3 (8.0 mg), 4 (11 mg), 5 (20 mg).

Compound **1** was isolated as a white powder. Its molecular formula was deduced as $C_{21}H_{24}O_4$ based on a prominent ion peak at *m/z* 363.1573 $[M + Na]^+$ (calc. 363.1572) in the HR-ESI-MS spectrum. The ^1H, ^{13}C NMR and HSQC spectral data indicated two sets of tetrasubstituted benzene groups [$\delta_{C/H}$ 105.4/6.50 (1H, d, *J* = 2.4 Hz, H-1), 98.0/6.51 (1H, d, *J* = 2.4 Hz, H-3), 127.1/7.89 (1H, d, *J* = 8.8 Hz, H-5), 113.9/6.59 (1H, d, *J* = 8.8 Hz, H-6)], three methylene groups [$\delta_{C/H}$ 29.8/2.88 (2H, dd, *J* = 16.4, 5.6 Hz, H-3′), 30.1/2.65 (2H, m, H-10), 24.5/2.57 (2H, m, H-9)], two methoxy groups [$\delta_{C/H}$ 55.9/3.78 (3H, s, 2-OMe), 55.5/3.80 (3H, s, 4-OMe)], two methyl groups [$\delta_{C/H}$ 26.1/1.29 (3H, s, -CH_3), 20.3/1.14 (3H, s, -CH_3)], and one methine group [$\delta_{C/H}$ 69.0/3.67 (1H, dd, *J* = 8.0, 5.6 Hz, H-2′)]. The above spectroscopic information indicated that **1** was a 9,10-dihydrophenanthrene derivative [10-11]. Its NMR spectral data was similar with spiranthol-C (Table.1) [7]. The distinction between **1** and spiranthol-C lies in that the quaternary carbon at C-4 of **1** is connected to a methoxy group (Figure1). Its ^1H-^1H COSY spectrum showed correlations between δ_H 7.89 (H-5) and δ_H 6.59 (H-6), δ_H 3.67 (H-2′) and δ_H 2.88 (H-3′), and δ_H 2.57 (H-9) and δ_H 2.65 (H-10), indicating the existence of three structural fragments (C-5 - C-6, C-2 ′ - C-3 ′, C-9 - C-10). The HMBC correlations from H-1 to C-2, C-3 and C-4a, from H-3 to C-1, C-2, C-4 and C-4a, from H-5 to C-7, C-4a, and C-8a, from H-6 to C-7, C-8, and C-4b, from H-9 to C-8, C-10, C-4b, C-8a, C-10a, from H-10 to C-1, C-8, C-9, C-8a and C-10a, from 2-OMe to C-2, from 4-OMe to C-4, from H-3′ to C-7, C-2′, C-4′ and C-8a, from H-5′ to C-2′, C-4′ and C-6′, from H-6′ to C-2′, C-4′ and C-5′ allowed the establishment of its planar structure (Figure 1). The *J* values of H-1 and H-3 is 2.4 Hz, indicating that H-1 and H-3 were meta-coupled aromatic. The locations of the two methoxy groups were determined by the HMBC correlations from 2-OMe to C-2, and from 4-OMe to C-4. In the HMBC spectrum, H-3 was correlated with C-4, but H-1 showed no correlation with C-4. Meanwhile, H-10 was correlated with C-1, but uncorrelated with C-3. Based on these analyses, the chemical shifts of C-1 and C-3 were distinguished. The HMBC correlation from H-5', H-6' to C-4', C-2' indicated that C-5', C-6' were connected to C-4', and C-4' was connected to C-2'. The HMBC correlations from H-3' to C-8, C-7, and C-8a showed that C-3' was connected to C-8. These HMBC correlations further confirmed the establishment of the planar structure.

The absolute configuration of C-2′ was determined to be *S* by comparing its ECD with calculated data (Figure 2). Therefore, compound **1** was determined to be (*S*)-2-(7,9-dimethyl-2,3,4,5-tetrahydrophenanthro [2,1-b] furan-2-yl) propan-2-ol, named Spiranthol B (Figure 1). Four known compounds, pleioanthrenin (2) [12], Sinensol B (3) [5], Spirasineol-A (4) [7], and Sinensol A (5) [12], were isolated and spectroscopically characterized, and their structures were determined by comparing the NMR and MS spectral data with those previously reported (Figure 3).

Bioactivity Test-Evaluation of the cell viability enhancing activities of **1**-5 *in vitro:* The bioactivity of compounds **1**-5 in promoting cell viability was evaluated in PA-induced apoptotic MIN6 cells. MIN6 cells culture and assay protocols were referenced with previous studies [13-14]. The abilities of compounds **1**-5 in enhancing the survival of MIN6 were tested by measuring the survival rate of pancreatic islet cells. Compound **1**-5, administered at two concentrations of 3.125, 6.25 μM, demonstrated a concentration-dependent enhancement of MIN6 cells survival, significantly improving viability compared to PA-injured cells (Figure 4). Compound 4 demonstrated a better enhancement in

cell viability when compared to other compounds. The results tentatively demonstrated that the isopentenyl moiety showed minimal impact on viability improvement. On the contrary, the presence of morel hydroxyl groups correlated positively with the enhancement of cell viability, indicating that an increased number of hydroxyl groups leads to better viability restoration.

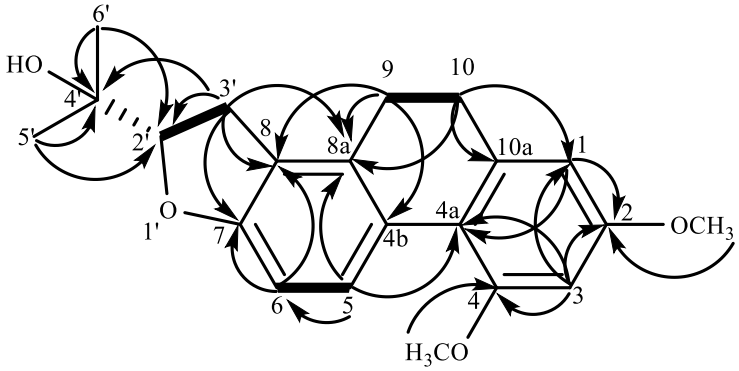

Figure 1. The structure, ^{1}H-^{1}H COSY (▬) and main HMBC (⤻) correlations of **1** add and between structure, ^{1}H-^{1}H COSY and HMBC

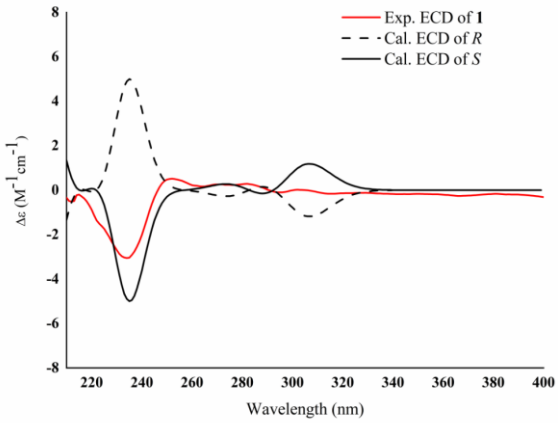

Figure 2. Experimental and calculated ECD spectra of **1**

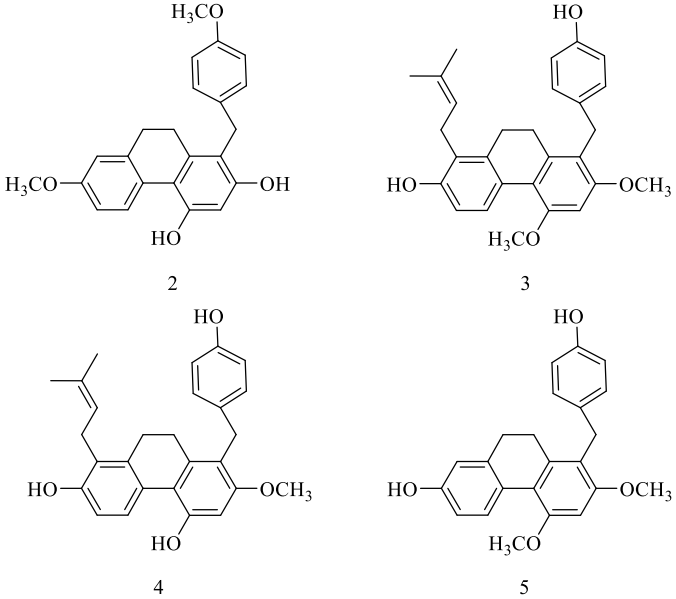

Figure 3. The structures of compounds 2-5

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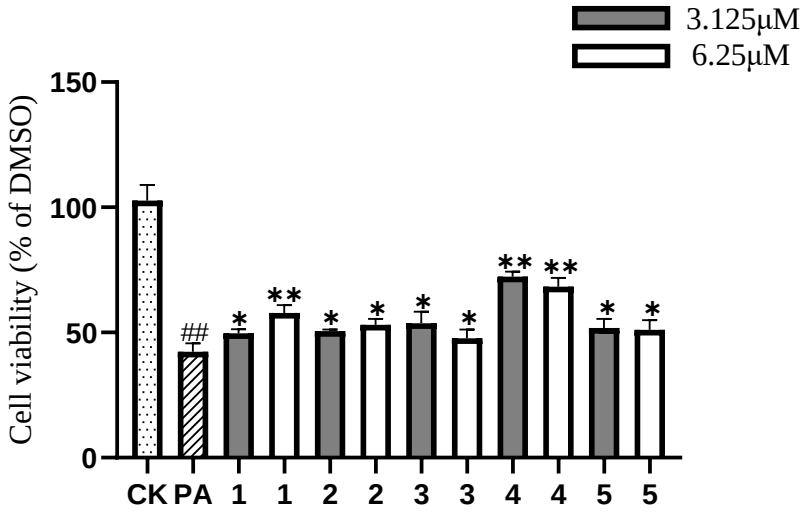

Figures 4. Effects of compounds **1**-5 on PA-induced MIN-6 cells viability
*p < 0.05, **p < 0.01 vs PA.; ##p < 0.01 vs. DMSO.

Table 1. ^{1}H NMR and ^{13}C NMR data for **1** and spiranthol-C(δ in ppm, *J* in Hz)

Position	spiranthol-C[a)]		**1** [b)]	
	δ_H	δ_C	δ_H	δ_C
1	6.36 (1H, *d*, *J* = 2.4)	106.7(CH)	6.50 (1H, *d*, *J* = 2.4)	105.4(CH)
2		158.6(C)		158.7(C)
3	6.42 (1H, *d*, *J* = 2.4)	100.8(CH)	6.51 (1H, *d*, *J* = 2.4)	98.0(CH)
4		153.3(C)		157.7(C)
4a		115.0(C)		116.4(C)
4b		125.2(C)		124.9(C)
5	7.80 (1H, *d*, *J* = 8.4)	106.7(CH)	7.89 (1H, *d*, *J* = 8.8)	127.1(CH)
6	6.72 (1H, *d*, *J* = 8.4)	125.5(CH)	6.59 (1H, *d*, *J* = 8.8)	113.9(CH)
7		157.9(C)		151.3(C)
8		125.2(C)		117.6(C)
8a		135.2(C)		137.2(C)
9	2.73 (2H, *m*)	26.2(CH_2)	2.57 (2H, *m*)	24.5(CH_2)
10	2.65 (2H, *m*)	30.2(CH_2)	2.65 (2H, *m*)	30.1(CH_2)
10a		140.6(CH)		140.2(C)
5'	1.37 (3H, *s*)	24.1(CH_3)	1.14 (3H, *s*)	20.3(CH_3)
6'	1.25 (3H, *s*)	26.33(CH_3)	1.29 (3H, *s*)	26.1(CH_3)
2-OMe	3.80 (3H, *s*)	55.3(CH_3)	3.78 (3H, s)	55.9(CH_3)
4-OMe			3.80 (3H, s)	55.5(CH_3)
2'	4.65 (1H, t, *J* = 8.9)	89.6(CH)	3.67 (1H, *dd*, *J* = 8.0, 5.6)	69.0(CH)
3'	3.14 (1H, d, *J* = 8.9)	29.8(CH_2)	2.88 (2H, *dd*, *J* = 16.4, 5.6)	29.8(CH_2)
4'		71.9(C)		76.5(C)

[a)] measured at 100 MHz in $CDCl_3$, [b)] measured at 150 MHz in DMSO-d_6

Acknowledgments

This work was funded by the Key Research and Development Program of Hainan Province (No. ZDYF2022SHFZ037) and the National Natural Science Foundation of China (82060778).

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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