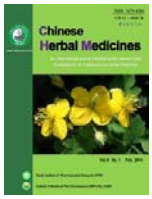

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Letter

A New Hasubanan Alkaloid from *Stephania hernandifolia*

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ABSTRACT

Objective To study the chemical constituents of *Stephania hernandifolia*. **Methods** Various chromato- graphic techniques were used to isolate the constituents, and the structures were elucidated on the basis of HR-EI-MS, 1D-NMR and 2D-NMR spectral analyses. **Results** A hasubanan alkaloid, hernsubanine D (**1**) was isolated from the whole plants of *S. hernandifolia*. The compound was screened for the cytotoxic activity against two human cancer cell lines *in vitro*. **Conclusion** Compound **1** is a new compound without cytotoxicity against A549 and K562 cells.

Key words

cytotoxicity; hasubanan alkaloid; hernsubanine D; Menispermaceae; *Stephania hernandifolia*

1. Introduction

Stephania hernandifolia (Willd.) Walp. (Menispermaceae) is a perennial twining vine mainly distributed in the southwest area of China, and used as a folk medicine for the treatment of rheumatoid arthritis, heatstroke, dysentery, mumps, sore throat, stomatitis, analgesia, and paralysis (State Administration of Traditional Chinese Medicine, 1999). The previous studies of this plant led to the isolation of some isoquinoline alkaloids such as hernandine (Semwal et al, 2010; Wang and Zhao, 1990; Kupchan et al, 1968). Recently, in the course of our investigation on the alkaloids in the plants of *Stephania* Lour. (Tang et al, 2013; 2010a; 2010b; He et al, 2010a; 2010b), a new hasubanan alkaloid, hernsubanine D (**1**) (Figure 1), was isolated from the whole plants of *S. hernandifolia*. *In vitro* experiments for the cytotoxic activity against two human cancer cell lines by an improved MTT method (Mizutani et al, 1995), compound **1** did not show any cytotoxicity against A549 and K562 cells. In this paper, we mainly described the isolation and structural elucidation of this new alkaloid by comparing its NMR data with that of the known hasubanan alkaloid stephabenine (**2**) (He et al, 2010b). In addition, the misassigned ^{13}C-NMR data of C-5, C-9, and C-15 of compound **2** were revised.

2. Materials and Methods

2.1 General

All solvents used for the extraction and isolation were distilled prior to use. The petroleum ether (60–90 ^{o}C) was used for the chromatography. For the column chromatography

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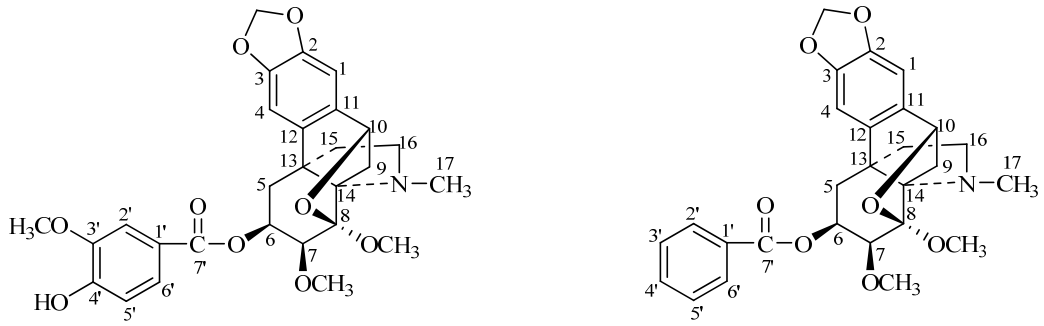

1 **2**

Figure 1 Structures of hernsubanine D (1) and stephabenine (2)

(CC), silica gel (SiO_2, 300–400 mesh, SiO_2 H 10–40 μm, Qingdao Marine Chemical, China), MCI gel CHP20P (75–150 μm; Mitsubishi Chemical Industries Company, Japan), Rp-18 gel (50 μm; YMC, Japan), and Sephadex LH-20 (40–70 μm; Amersham Pharmacia Biotech AB, Sweden) were used. TLC was performed on precoated GF_{254} silica gel plates (Qingdao Marine Chemical, China) and spots were visualized by spraying the Dragendorff reagent. X–4 Melting-point Apparatus, Rudolph Autopol 1 Digital Polarimeter (2.5 cm cell), Shimadzu UV–2401 PC UV/VIS Spectrophotometer, Bruker Tensor 27 FT-IR Spectrometer, Bruker DPX–500 NMR Spectrometer, and VG Auto Spec–3000 Mass Spectrometer were used as well.

2.2 Plant materials

The whole plant of *Stephania hernandifolia* (Willd.) Walp. was collected in Luodian (Guizhou, China) in August 2008, and identified by Prof. An-ren Li, Institute of Botany, Chinese Academy of Sciences. A voucher specimen (batch No. Zhang 20080813) has been deposited in the Key Laboratory of Chemistry for Natural Products of Guizhou province and Chinese Academy of Sciences.

2.3 Extraction and isolation

The dried and powdered whole plant (22.0 kg) of *S. hernandifolia* was extracted by 95% EtOH refluxing (100 L × 4) for four times, and 3 h for each time. After removing the solvent under reduced pressure, the residue (1020 g) was partitioned between petroleum ether and 5% HCl. The aqueous phase was adjusted to pH 7 with saturated NH_3-H_2O and extracted with $CHCl_3$ to give crude alkaloid (490 g). The crude alkaloid was subjected to silica gel CC eluted with $CHCl_3$-MeOH (100:0→0:100) to yield 12 fractions (Frs. A–L). Fr. B (57.0 g) ($CHCl_3$-MeOH 100:2) was isolated by MCI gel CC with MeOH-H_2O (30:100→100:0) to afford four fractions (Subfrs. B_1–B_4). Subfr. B_4 (0.7 g) (MeOH-H_2O 90:100) was further separated by repeated Rp-18, Sephadex LH-20, and silica gel CC using MeOH-H_2O (90:100 and 70:100), $CHCl_3$-MeOH (1:1), and $CHCl_3$-AcOEt (8.5:1.5) to obtain compound **1** (42 mg).

3. Results and discussion

Hernsubanine D (**1**) (6β,7β,8β,10β)-8,10-epoxy-7,8-dimethoxy-2,3-[methyl-enebis(oxy)]-hasubanan-6-[(*Z*)-3-hydroxyl-4-methoxyl-cinnamate]. White crystal (MeOH). mp 198–200 °C. $[\alpha]_D^{24}$ 2.7 (*c* 0.50, $CHCl_3$). UV $\lambda_{max}^{CHCl_3}$ (nm): 257 (3.05), 290 (2.93), 485 (1.53). IR ν_{max}^{KBr} (cm^{-1}): 3430, 2924, 2852, 1697, 1613, 1539, 1284, 1034. EI-MS *m/z*: 525 $[M]^+$, 228, 213, 168, 151. HR-EI-MS: 525.1979 ($[M]^+$, $C_{28}H_{31}NO_9^+$; calc. 525.1999). ^{1}H-NMR and ^{13}C-NMR data are shown in Table 1.

Hernsubanine D (**1**) was obtained as colorless crystal. Its molecular formula was assigned as $C_{28}H_{31}NO_9$ by HR-EI-MS at *m/z* 525.1979 ($[M]^+$, calc. 525.1999), with 14 degrees of unsaturation. The UV absorption recorded at 257 (3.05), 290 (2.93), 485 nm (1.53) implied the presence of a highly conjugated system in compound **1**. The IR spectrum of compound **1** suggested the absorption bands of OH (3430 cm^{-1}), conjugated C=O group (1697 cm^{-1}), and aryl group (1613, 1539, and 1284 cm^{-1}). The ^{13}C-NMR spectrum consisted of 28 signals corresponding to four methyls (three oxygenated and one aminated), five methylenes (four saturated and one methylenedioxy), eight methines (five aromatic and three saturated) groups, and eleven quaternary carbons (one C=O, seven aromatic, and three saturated). Combined with the typical EI-MS fragmentation ions at *m/z* 228 and 168, ^{13}C-NMR data at δ 103.2 (s) and 77.2 (d), compound **1** was deduced to be a hasubanan-type alkaloid with a ketal ether bridge between C-8 and C-10 (Zhang and Yue, 2005). The ^{1}H-^{1}H COSY and HMQC spectra revealed the presence of a methylenedioxy group and isolated -CH_2CHORCHOR-, -CH_2CHOR-, and -CH_2CH_2- fragments (Figure 2). Further examination of the ^{1}H-NMR, ^{13}C-NMR, and 2D-NMR data and the degrees of unsaturation of compound **1** suggested that compound **1** was similar to the known alkaloid stephabenine (Figure 1) (Yamamura and Matsui, 1985) except for the substituent groups at C-6. In compound **1**, the ester group was determined to be a vanillate group as judged by ^{1}H-NMR and ^{13}C-NMR data (Table 1), and confirmed by HMBC correlations of H-2′ with C-1′, C-3′, C-4′, C-6′, C-7′, H-5′ with C-1′, C-2′, C-3′, C-4′, C-7′, H-6′with C-2′, C-3′, C-4′, C-7′, and MeO-C-3′ with C-3′ (Figure 2).

Table 1 NMR data of compound 1 and stephabenine

Positions	Compound **1**[a]		Stephabenine[b]
	δ_H	δ_C	δ_C
1	6.43 (s, 1H)	106.9 (d)	107.0
2	–	147.7 (s)	147.7
3	–	144.6 (s)	144.7
4	6.55 (s, 1H)	106.0 (d)	106.0
5	1.83 (m, 2H)	37.3 (t)	37.5
6	5.51 (brs, 1H)	67.7 (d)	68.0
7	3.79 (brs, 1H)	81.4 (d)	81.6
8	–	103.2 (s)	103.4
9	2.69 (m, 1H)	29.7 (t)	29.4
	1.54 (d, J = 9.9 Hz, 1H)		
10	4.89 (d, J = 6.0 Hz, 1H)	77.2 (d)	77.1
11	–	137.0 (s)	137.0
12	–	133.2 (s)	133.5
13	–	49.5 (s)	49.7
14	–	75.5 (s)	77.1
15	2.37 (m, 2H)	36.5 (t)	36.7
16	3.34 (m, 1H), 2.53 (m, 1H)	53.9 (t)	53.9
17	2.56 (s, 3H)	38.6 (q)	38.6
1'	–	121.8 (s)	129.6
2'	7.29 (br, s, 1H)	111.7 (d)	129.9
3'	–	145.6 (s)	127.5
4'	–	149.7 (s)	132.3
5'	6.68 (d, J = 8.1 Hz, 1H)	113.2 (d)	127.5
6'	6.54 (brd, J = 8.1 Hz, 1H)	125.0 (d)	129.9
7'	–	166.2 (s)	166.3
7-OMe	3.41 (s, 3H)	57.6 (q)	57.7
8-OMe	3.54 (s, 3H)	51.6 (q)	51.6
3'-OMe	3.89 (s, 3H)	56.1 (q)	–
$-OCH_2O-$	5.74, 5.29 (d, J = 1.2 Hz, 2H)	100.7 (t)	100.6

[a] ^{1}H-NMR and ^{13}C-NMR were measured in $CDCl_3$ at 500 and 125 MHz.
[b] see Yamamura and Matsui (1985)

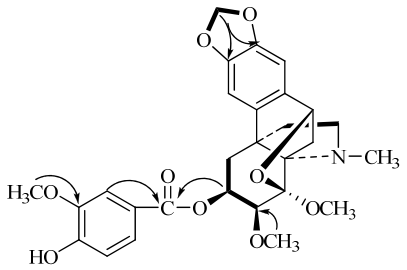

Figure 2 ^{1}H-^{1}H COSY (–) and key HMBC (H→C) correlations of compound 1

Therefore, compound **1** was identified as (6β,7β,8β,10β)-8,10-epoxy-7,8-dimeth-oxy-2,3-[methylenebis(oxy)]-17-methyl-hasubanan-6-vanillate and confirmed by the HSQC, HMBC, and ^{1}H-^{1}H COSY spectra. Meanwhile, the ^{13}C-NMR data of C-5, C-9, and C-15 of stephabenine were revised.

Acknowledgments

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