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Original Article

Quinoline alkaloids isolated from *Scolopendra subspinipes mutilans*

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ABSTRACT

Objective: To study the quinoline alkaloids from the ethanol extract of *Scolopendra subspinipes mutilans* (SSM).
Methods: The chemical constituents were isolated and purified by macroporous resin column, medium pressure preparation chromatography, and semi-preparative HPLC. Their structures were elucidated by IR, MS, and NMR experiments.
Results: Three quinolone alkaloids were obtained and identified as 3-hydroxy-4-methoxyquinolin-8-yl hydrogen sulfate (**1**), jineol-8-sulfate (**2**), and jineol (**3**), respectively.
Conclusion: Compound **1** is a new compound from SSM.
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1. Introduction

Centipede (Wugong in Chinese), the dry body of *Scolopendra subspinipes mutilans* L. Koch (SSM), has been recorded earliest in the book *Shennong's Herbal Classic* on the medicinal use. In China, this medicine has been widely used with the form of crude drug powder or water extract to treat convulsions, tetanus, apoplexy, tumor, and pertussis in children. (Jiangsu New Medical College, 1986). There is only one species, i.e. SSM, recorded in the Chinese Pharmacopoeia, but other species are also used in some regions of China. The genus *Scolopendra* is mainly composed of proteins, peptides, amino acids, lipids, and trace elements, but most animal-derived medicines contain these similar ingredients. In recent years, scholars paid much attention to the constituents of quinoline alkaloids in SSM, including jineol (Moon et al., 1996), scolopendrine (Noda, Yashiki, Nakatani, Miyahara, & Du, 2001), 2,8-dihydroxy-3,4-dimethoxyquinoline (Yoon et al., 2006), 3,5-dihydroxyquinoline (Liu, Nie, Sun, & Liu, 2016), and jineol-8-sulfate (Lee et al., 2016). Among them, jineol showed certain activity *in vitro* against several human tumor cells and antioxidant effect against LDL-oxidation has been used as a quantitative reference for centipedes (Liu, Fan, Li, Su, & Zhang, 2017). In our present research, three quinoline alkaloids (Fig. 1) were obtained from SSM, and compound 1 was found to be a new compound, named as 3-hydroxy-4-methoxyquinolin-8-yl hydrogen sulfate. This paper reports the isolation and structural elucidation of 3-hydroxy-4-methoxyquinolin-8-yl hydrogen sulfate in detail.

2. Materials and methods

2.1. Apparatus and reagents

The IR spectra were recorded by a Shimadazu FTIR-8400S infrared spectrophotometer (Shimadazu, Kyoto, Japan) in KBr pellets. The NMR spectra were measured on a Varian Inova 600 MHz spectrometer (Palo Alto, USA) with TMS as the internal standard. HR-ESI-MS were carried out on an Agilent 6520 Q-TOF mass spectrometer (Agilent Technologies, Palo Alto, USA). Separation and purification were performed by medium pressure preparation chromatography (Lisui Sceince, Shanghai, China) and Agilent 1100 liquid chromatograph (USA) equiped with a Variant semi-preparative C_{18} column (250 mm × 21 mm, 5 μm). Silica gel (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, China) and macroporous resin (XDA-8, Cangzhou Bon Chemical Co., Ltd., Cangzhou, China) were also used for column chromatography. HPLC-grade acetonitrile was provided by Tedia Company, Inc. The other solvents used were of analytical grade.

2.2. Experimental materials

The centipede was purchased from Anguo Herbal Market, Hebei Province, in May 2013. The species was authenticated to be the dried body of *S. subspinipes mutilans*, the official source of medicinal Wugong, by professor Shou-xin Li from Lunan Pharmaceutical

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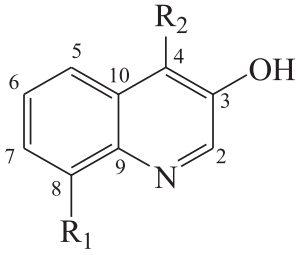

1 R_1=OSO_3H R_2=OCH_3
2 R_1=OSO_3H R_2=H
3 R_1=OH R_2=H

Fig. 1. Chemical structures of compounds **1**–**3**.

Group Co., Ltd. A voucher specimen (LN 0130511) has been deposited at the State Key Laboratory of Generic Manufacture Technology of Traditional Chinese Medicine.

2.3. Extraction and isolation

The dried body of SSM (1 kg) was extracted with 70% ethanol (2 × 10 L) at room temperature and the solvent was removed by vacuum rotary evaporator and partitioned between H_2O and EtOAc. The water fraction (301.5 g) was subjected to macroporous resin column chromatography eluted with 50% EtOH. After concentration, the residue was purified by medium pressure preparation chromatography and semi-preparative HPLC using acetone-0.1% phosphoric acid solution (5:95, 20 mL/min) to yield compound **1** (43 mg) and compound **2** (126 mg). After being degreased by petroleum ether, the EtOAc extract (10.1 g) was applied to silica gel column eluted with $CHCl_3$-MeOH (10:1) to obtain Fr. B (2.3 g). Fr. B was dissolved in mobile phase, and further separated by medium pressure preparation chromatography eluted with acetone-10 mmol/L KH_2PO_4 (20:80) to give compound **3** (185 mg). The process of the extraction and isolation was monitored by HPLC (Fig. 2), and the mobile phase consisted of a water solution containing 10 mmol/L potassium dihydrogen phosphate and acetonitrile with gradient elution.

3. Results

3.1. Spectroscopic data

Compound **1**: pale-yellow powder. IR (KBr): υ_{max} = 3221.80, 1598.23, 1568.36, 1344.45, 1313.00, 1246.83, and 1174.89 cm^{-1}. ^1H NMR (DMSO–d_6, 400 MHz) and ^{13}C NMR (DMSO–d_6, 100 MHz) were shown in Table 1. HR-ESI-MS calcd for $C_{10}H_9NO_6S$ Na $[M+Na]^+$ 294.0043, found 294.0044.

Compound **2**: white powder. IR (KBr): υ_{max} = 3532.97, 1573.72, 1563.59, 1350.24, 1319.76, 1184.73, 1237.36, 1046.55, 759.89 cm^{-1}. ^1H NMR (DMSO–d_6, 400 MHz) and ^{13}C NMR (DMSO–d_6, 100 MHz) are shown in Table 1. ESI-MS *m/z*: 264.0 $[M+Na]^+$, ESI-MS *m/z*: 240.0 $[M-H]^-$.

Compound **3**: yellow powder. ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) are shown in Table 1. HR-ESI-MS calcd for $C_9H_7NO_2$ $[M+H]^+$ 163.0581, found 162.055 0.

3.2. Structure elucidation

Compound **1**: Its molecular formula was determined to be $C_{10}H_9NO_6S$ with seven degrees of unsaturation on the basis of the HR-ESIMS (positive ion) *m/z* 294.0044 $[M+Na]^+$ (294.0043 calcd for $C_{10}H_9NO_6S$ Na) and the ^{13}C NMR data. The IR spectrum (KBr pellet) showed absorption bands at 3221, 1598, 1568, 1344, 1313, 1246, and 1174 cm^{-1} ascribable to a hydroxyquinoline moiety group and a sulfate group. The ^1H NMR spectrum showed signals due to one methoxyl group (δ 4.38) and four aromatic proton signals. Comparing the data with those reported in the literature (Ding, Guo, Wu, Qi, & Xu, 2016; Tang, Zhi, & Tu, 1995),

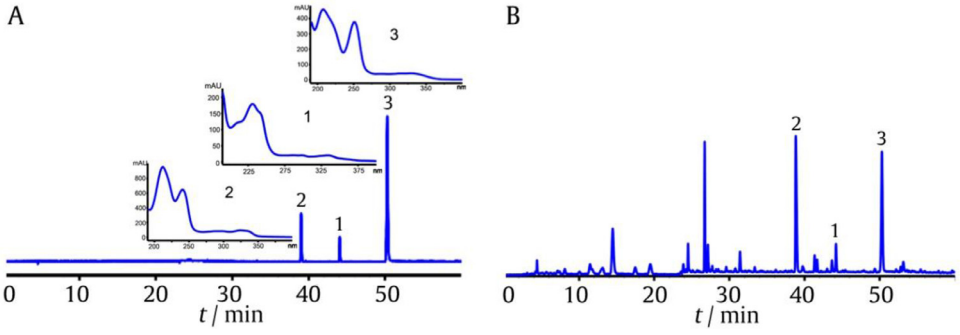

Fig. 2. HPLC chromatogram of mixed compounds (A) and ethanol extract of SSM (B). 1: 3–hydroxy-4-methoxyquinolin-8-yl hydrogen sulfate; 2: jineol-8-sulfate; 3: jineol.

Table 1
NMR data of compound **1**, **2**, and **3** (δ in ppm, *J* in Hz).

Positions	1		2		3	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2	8.60 (1H, s)	138.66	8.52 (1H, d, 2.8 Hz)	142.90	8.46 (1H, d, 3.0 Hz)	140.90
3		141.36		151.39		151.59
4		153.87	7.44 (1H, d, 2.8 Hz)	115.64	7.40 (1H, d, 2.4 Hz)	115.94
5	7.88 (1H, d, 8.0 Hz)	116.45	7.42 (1H, dd, 8.4, 2.0 Hz)	120.73	7.11 (1H, d, 8.4 Hz)	116.50
6	7.68 (1H, t, 8.0 Hz)	128.70	7.39 (1H, t, 7.2 Hz)	127.19	7.27 (1H, t, 7.8 Hz)	127.66
7	7.85 (1H, d, 8.0 Hz)	119.65	7.63 (1H, dd, 7.2, 2.0 Hz)	114.63	6.86 (1H, d, 7.2 Hz)	107.70
8		145.48		150.04		152.75
9		130.34		135.73		133.40
10		124.70		130.73		130.45
OCH_3	4.38 (3H, s)	62.21				

NMR: compound **1** and **2**, 400 and 100 MHz in DMSO–d_6; compound **3**, 600 and 150 MHz in CD_3OD.

the value of δ 4.38 was similar with 1-methoxyl group at δ 4.43 in 1–methoxy-4,5-diolisoquinoline and 4-methoxyl group (δ 4.45) in dictamnine. Both *o*-position and *p*-position of the methoxyl group have electron-withdrawing groups, which cause chemical shift downfield. Hence, these observations indicated the existance of a methoxyl group at δ 4.38 (3H, s). The ^{13}C NMR and HSQC spectra indicated the presence of four methines at δ 138.66, 128.70, 119.65, and 116.45; Five quaternary carbons at δ 153.87, 145.48, 141.36, 130.34, and 124.70; And a methoxyl carbon at δ 62.21 attached to δ 153.87 (C-4). The NMR spectra were closely matched to those of jineol (**3**), while the proton of H-7 shifted downfield significantly and the carbon atom at δ 145.48 (C-8) shifted upfield 6.27 relative to jineol. These evidences indicated the presence of a sulfate group at C-8.

In the HMBC experiment (Fig. 3), the key correlations from the proton δ 8.60 (1H, s) to the carbon at δ 153.87 (C-4), 130.34 (C-9), and 141.36 (C-3); from the proton δ 7.88 (1H, d, J = 8.0 Hz) to δ 153.87 (C-4), 130.34 (C-9), and 124.70 (C-10); from the proton δ 7.85 (1H, d, J = 8.0 Hz) to δ 130.34 (C-9) and 145.48 (C-8). The methoxyl proton (δ 4.38) gave a correlation with C-4 carbon (δ 153.87). Meanwhile, the correlations of H-5/H-6/H-7 were observed in the ^{1}H–^{1}H COSY spectrum. From the information described above, the structure of compound **1** was established to be 3–hydroxy-4-methoxyquinolin-8-yl hydrogen sulfate (Fig. 1).

From the data of IR, NMR, and MS and comparing with those from literatures, Compounds **2** and **3** were identified as jineol-8-sulfate and jineol. The NMR spectral data of these compounds were listed in Table 1.

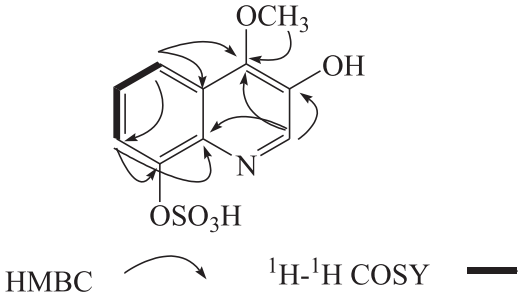

Fig. 3. Key HMBC and ^{1}H–^{1}H COSY correlations of compound **1**.

4. Discussion

Centipedes are toxic animals, so the misuse among species can cause potential harm to human health, although it is difficult to identify these species because of the similarity in physical characteristics. We also collected other species, e.g. *S. multidens* Newport (LN 0130512), *S. mojiangica* Zhang et Chi (LN 0130513), and *S. negrocapitis* Zhang et Wang (LN 0130514) in 2013 and compared the differences of compounds **1**–**3** in these three species. It is found that the content of compound 1 was the highest in *S. multidens* Newport, less in SSM, and the least even almost none in *S. mojiangica* and *S. negrocapitis*. Hence, the compound **1** could be used as an important marker for the genus *Scolopendra* to identify these species.

Conflict of Interest

There is no any conflict of interest.

Acknowledgments

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