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CYTOTOXIC COMPOUNDS FROM *SCOLOPENDRA SUBSPINIPES MUTILANS*

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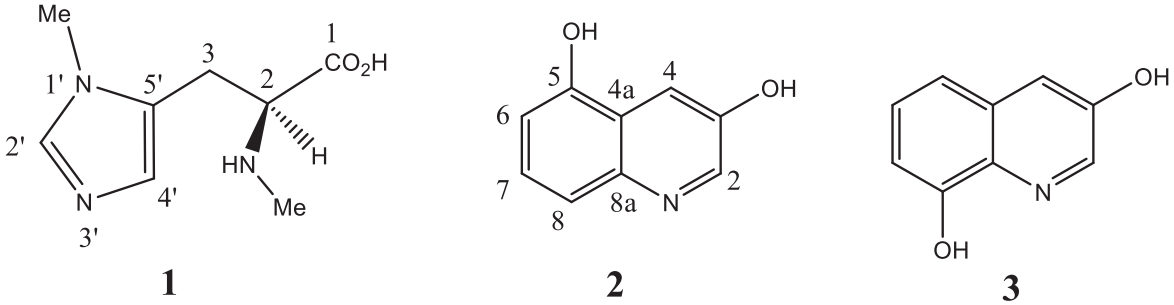
Abstract – A new amino acid, 2*S*-3-(1-methyl-1*H*-imidazol-5-yl)-2-(methylamino)propanoic acid (**1**) and a new natural product, 3, 5-dihydroxyquinoline (**2**), along with a known compound (**3**) were isolated from the centipede *Scolopendra subspinipes mutilans* L. Koch. Their structures were elucidated on the basis of extensive one-dimensional (1D)- and 2D-NMR spectroscopic analyses and mass spectrometry. All isolates were evaluated for their cytotoxic activities against three human cancer cell lines, HepG-2, HT-29, and A549. Compounds **2** and **3** exhibited moderate cytotoxic activities with IC_{50} values of 1.95–27.20 μM against the three cancer cell lines.

The centipede, *Scolopendra subspinipes mutilans* L. Koch (Scolopendridae), has been utilized as a traditional Chinese medicine for the treatment of endogenous liver wind, spasm, childhood convulsion, and tetanus.[1] In addition, it was found to exhibit the anticancer activities.[2,3] More researchers paid much attention to the constituents of protein, especially of venom,[4-11] whereas the effective small molecules are far from enough. To date only five small molecules have been isolated from the centipede,[12-15] in which no more than one associated with the antitumor activity has been reported.[12]



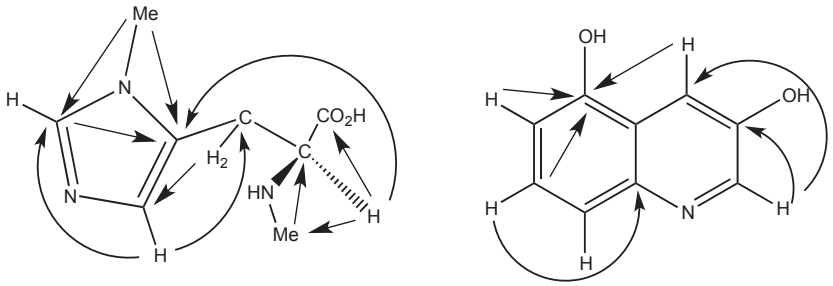
1 **2** **3**

Figure 1. Chemical Structures of Compounds **1**–**3**

In the search for cytotoxic constituents from this crude drug, a new amino acid (**1**) and a new natural product (**2**), together with a known compound (**3**) were isolated from *S. subspinipes mutilans* (**Figure 1**). Herein, we reported the isolation and structure elucidation of these compounds, as well as their cytotoxic activities against HepG-2, HT-29, and A549 cell lines.

Repeated column chromatography (silica gel, RP-18, and MPLC) of the MeOH extract of *S. subspinipes mutilans* L. Koch resulted in the isolation of three compounds (**1**–**3**). The chemical structures of the known compound were identified as jineol (**3**) by comparing their spectroscopic data with those reported in the literature.[12]

Compound **1** was isolated as a white solid with the molecular formula $C_8H_{13}N_3O_2$ as determined by high-resolution electrospray ionization mass spectra (HR-ESI-MS) $[M+H]^+$ *m/z* 184.1094 (Calcd 184.1086 for $C_8H_{14}N_3O_2$), and was positive to ninhydrin reagent. The ^{1}H NMR spectrum of **1** revealed the presence of two N-methyl group at δ_H 3.67 (3H, s) and δ_H 2.72 (3H, s); two aromatic protons at δ_H 7.79 and 7.00; two sets of alkyl signals at δ_H 3.79 (1H, t, J = 6.4 Hz) and δ_H 3.26 (2H, d, J = 6.4 Hz). In accordance with the molecular formula, 8 carbon signals were resolved in ^{13}C NMR spectra of **1**, with aid of the HSQC experiments, assignable to two methyls, one methylene, three methines (one nitrided and two aromatic), and two quaternary carbons (one aromatic and one carboxyl). The ^{1}H–^{1}H COSY spectrum indicated the presence of one proton–proton sequence (–$C_{(2)}$H–$C_{(3)}H_2$–). The HMBC correlations from δ_H 3.79 (H-2) to N-methyl carbon δ_C 34.9 and from N-methyl proton at δ_H 2.72 to δ_C 64.9 (C-2) indicated the N-methyl group was located at the C-2 position (**Figure 2**), which was also verified by the nuclear Overhauser effect (NOE) correlation from N-methyl proton at δ_H 2.72 to δ_H 3.79 (H-2) and δ_H 3.26 (H-3) (**Figure 3**). The HMBC correlations from δ_H 3.79 (H-2) and δ_H 3.26 (H-3) to the carboxyl carbon δ_C 175.2 showed the carboxyl group also existed at the C-2 position. All these revealed the existence of one 2-(methylamino)propanoic acid unit.



1 **2**

Figure 2. Key HMBC Correlations of **1** and **2**

The remaining elements comprised one methyl, two methylenes, one quaternary carbon and two nitrogen atoms representing a methylated imidazole substructure. The methyl (δ_H 3.67; δ_C 34.2) located at the $N_{1'}$

was found by the HMBC correlation from δ_H 3.67 to δ_C 141.9 (C-2'), δ_C 129.2 (C-5') (**Figure 2**), and by the NOE correlation between δ_H 3.67 and δ_H 7.79 (H-2'), δ_H 3.26 (H-3) (**Figure 3**). The HMBC spectrum showed the correlations from δ_H 3.79 (H-2) to δ_C 129.2 (C-5'), as well as from δ_H 3.26 (H-3) to δ_C 128.4 (C-4') (**Figure 2**). In addition, the cross-peak in its nuclear Overhauser effect spectroscopy (NOESY) spectrum from δ_H 3.26 (H-3) to δ_H 7.00 (H-4') and $N_{1'}$-methyl group (δ_H 3.67) was also observed (**Figure 3**). These results showed that C-3 (δ_C 26.8) of the 2-(methylamino)propanoic acid unit was directly attached to C-5' (δ_C 129.2) of the imidazole substructure. The absolute configuration at C-2 was deduced to be *S* according to its biogenesis and by comparison of its specific rotation, $[\alpha]_D^{25}$ -12.6 (*c* 1.9, H_2O) with those of L(*S*)-histidine, $[\alpha]_D^{25}$ -39.7 (*c* 1.13, H_2O).[16] Thus, **1** was established as 2*S*-3-(1-methyl-1*H*-imidazol-5-yl)-2-(methylamino)propanoic acid.

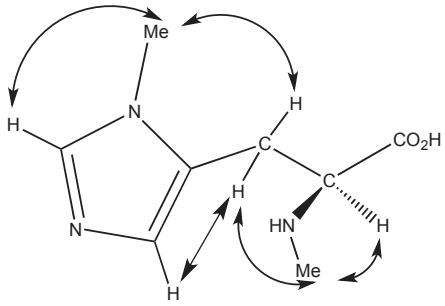


Figure 3. Key NOESY Correlations of **1**

Compound **2** was obtained as a yellowish amorphous powder, and gave a greenish-yellow fluorescence spot under UV_{254} light. Its molecular formula was deduced to be $C_9H_7NO_2$ by the HR-ESI-MS (*m/z* 162.0550 $[M+H]^+$ Calcd for $C_9H_8NO_2$, 162.0555), indicating 7 degrees of unsaturation. The UV spectrum showed absorption maxima at 215 and 245 nm. The ^{13}C NMR spectrum of **2** revealed the presence of nine aromatic carbons. Furthermore, distortionless enhancement by polarization transfer (DEPT, 90 and 135) experiments showed 9 carbon resonances, consisting of five methine at δ_C 117.7, 117.9, 124.0, 128.3 and 144.4, and four quaternary carbons at δ_C 132.6, 136.7, 149.3 and 153.2. These observations suggest a hydroxyquinoline moiety. The methine protons were determined to be H-2, H-4, H-6, H-7, and H-8 protons by a combination of ^1H NMR spectrum and ^1H–^1H correlation spectroscopic (COSY) experiments. The methine proton (d, J = 2.7 Hz, H-2) at δ 8.54 was coupled to the methine proton (d, J = 2.7 Hz, H-4) at δ 7.52. The methine proton (dd, J = 7.6, 1.2 Hz, H-6) at δ 7.71 was strongly coupled to the methine proton (t, J = 7.6 Hz, H-7) at δ 7.46, which in turn was coupled to the methine proton (dd, J = 7.6, 1.2 Hz, H-8) at δ 7.53.

There were correlations between C-2 (δ_C 144.4) and H-2 (δ_H 8.54); C-4 (δ_C 117.9) and H-4 (δ_H 7.52); C-6 (δ_C 117.7) and H-6 (δ_H 7.71); C-7 (δ_C 128.3) and H-7 (δ_H 7.46); C-8 (δ_C 124.0) and H-8 (δ_H 7.53) based

on the heteronuclear single quantum coherence (HSQC) experiments. In the heteronuclear multiple bond connectivity (HMBC) experiments (**Figure 2**), the carbon signal (C-3) at δ 153.2 showed correlations with δ_H 8.54 (H-2), and the carbon signal (C-5) at δ 149.3 with the proton signals (H-4, H-6, H-7) at δ 7.52, 7.71, and 7.46, respectively. Key correlations were also shown between C-4a and H-6; C-8a and both H-7 and H-8; C-4 and H-2. Above all, the structure of **2** was confirmed to be 3, 5-dihydroxyquinoline. Although it was previously reported in the organic synthetic studies,[17,18] this is the first report of its occurrence in nature, and its structure bearing oxygen at the 3-position is very unique.

Table 1. Cytotoxic Activities of Compounds (**1**–**3**)

Compounds	IC_{50} (μM)		
	HepG2	HT-29	A549
1	> 50	> 50	> 50
2	1.93±0.25	23.79±2.44	18.43±2.80
3	6.81±0.72	27.20±3.06	11.62±1.59
taxol	0.007±0.001	0.020±0.003	0.033±0.006

The isolated compounds were tested for cytotoxicity *in vitro* by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in three human tumor cell lines: HepG2 liver cancer cells, HT-29 colon cancer cells, and A549 lung cancer cells. As shown in **Table 1**, compounds **2** and **3** displayed moderate cytotoxic activities with IC_{50} values of 1.95–27.20 μM against three cancer cell lines, whereas compound **1** showed no cytotoxic activity (IC_{50} > 50 μM) against all tested cancer cell lines. Among them, **2** exhibited the highest activity with an IC_{50} value of 1.93 μM against HepG-2. Additionally, it was found that compounds **2** and **3** all showed the higher activities against HepG-2 cell lines than those against HT-29 and A549, which might be associated with what the meridian tropism of *S. subspinipes mutilans* is in the liver. These results indicated the quinoline alkaloids may contribute to the anticancer effect of this folk medicine, which would be further investigated in the future. Further work on other biological activities of compound **1** is in progress.

EXPERIMENTAL

General. Optical rotation was measured on a Rudolph Autopol IV polarimeter. UV spectrum was taken in MeOH using a Hitachi U-3310 spectrophotometer. HR-ESI-MS were obtained on a Bruker micrOTOF-Q II spectrometer. ^{1}H- and ^{13}C-NMR spectra were acquired on a Bruker Avance III 400 spectrometer. ^{1}H-^{1}H COSY, NOESY, HSQC and HMBC spectra were recorded using standard Bruker programs. CC was performed with silica gel (300-400 mesh, Qingdao Haiyang Chemical Group Co., Ltd., PR China), reversed-phase C_{18} silica gel (YMC, Japan). MPLC was carried out on a BUCHI apparatus

equipped with a C-605 pump. TLC analyses were conducted on precoated TLC sheets of silica gel 60 GF254 (Qingdao Haiyang Chemical Group Co., Ltd.), detected under a UV lamp at 254 nm and visualized by spraying 0.2% ninhydrin in EtOH.

Insect Material. The dried centipede, *S. subspinipes mutilans*, was collected in Anguo county of Hebei province, China, in May 2013 and identified by Professor Qing-Hua Liu, Xinjiang Institute of Materia Medica. A voucher specimen (HB-13-0529) has been deposited at the Department of Pharmacy Engineering, Tianjin University of Technology.

Extraction and Isolation. The dried whole bodies (1.9 kg) of centipedes were extracted twice with MeOH. After filtration, the MeOH solvent was evaporated to give a residue (640 g). The MeOH extracts (640 g) were chromatographed on a silica-gel column chromatography eluting with a gradient of petroleum ether–EtOAc (20 : 1–10 : 1) and CH_2Cl_2–MeOH (20 : 1–0 : 20) to afford seven fractions (C1–C7) based on TLC pattern. Fraction C6 (7.02 g) was subjected to a silica gel column chromatography eluting with a gradient of CH_2Cl_2–MeOH (5 : 1–5 : 5) to afford thirteen subfractions 12.1–C12.13. The subfraction C12.10 (268.3 mg) was subjected to a silica-gel column chromatography eluting with CH_2Cl_2–MeOH (2 : 1) to afford compound **1** (22.6 mg). Fraction C2 (24.35 g) was subjected to column chromatography on silica gel eluted with CH_2Cl_2–MeOH (20 : 1) repeatedly to give a brown residue (0.39 g), which was loaded on a RP C_{18} MPLC column eluted with MeOH–H_2O (1 : 2) to obtain compound **3** (10.4 mg). Fraction C5 (2.33 g) was subjected to column chromatography on silica gel eluted with CH_2Cl_2–MeOH (7 : 1) repeatedly, and then purified by RP C_{18} MPLC chromatography eluted with MeOH–H_2O (6 : 1) to yield compound **2** (5.8 mg).

Compound 1: white solid; $[\alpha]_D^{25}$ -12.6 (*c* 1.9, H_2O); ^{1}H NMR (D_2O, 400MHz) δ 7.79 (part of 1 H was exchanged with D_2O, s, H-2'), 7.00 (1 H, s, H-4'), 3.79 (1 H, t, J = 6.4 Hz, H-2), 3.67 (3 H, s, $N_{1'}$-CH_3), 3.26 (2 H, d, J = 6.4 Hz, H-3), 2.72 (3 H, s, NH-CH_3); ^{13}C NMR (D_2O, 100 MHz) δ 175.2 (s, C-1), 141.9 (d, C-2'), 129.2 (s, C-5'), 128.4 (d, C-4'), 64.9 (d, C-2), 34.9 (q, NH-CH_3), 34.2 (q, $N_{1'}$-CH_3), 26.8 (t, C-3); HMBC correlations: H-2 to C-1, C-3, -NH*C*H$_3$, C-5'; H-3 to C-1, C-2, C-4'; NH-C*H*$_3$ to C-2; H-2' to C-5'; H-4' to C-2', C-5', C-2, C-3; $N_{1'}$-C*H*$_3$ to C-2', C-5'; NOESY correlations: H-2 to H-3, H-4', -NHC*H*$_3$; H-3 to H-2, H-4', -NHC*H*$_3$, $N_{1'}$-C*H*$_3$; -NHC*H*$_3$ to H-2, H-3, H-4'; H-2' to $N_{1'}$-C*H*$_3$; H-4' to H-2, H-3, -NHC*H*$_3$; $N_{1'}$-C*H*$_3$ to H-2'; HR-ESI-MS (positive mode) *m/z* 184.1094 $[M+H]^+$ (calcd 184.1086 for $C_8H_{14}N_3O_2$), 367.2086 $[2M+H]^+$ (calcd 367.2094 for $C_{16}H_{27}N_6O_4$).

Compound 2: yellowish amorphous powder; UV λ_{max} (MeOH) nm: 215, 245; ^{1}H NMR (CD_3OD, 400MHz) δ 8.54 (1 H, d, J = 2.7 Hz, H-2), 7.71 (1 H, dd, J = 7.6, 1.2 Hz, H-6), 7.53 (1 H, dd, J = 7.6, 1.2 Hz, H-8), 7.52 (1 H, d, J = 2.7 Hz, H-4), 7.46 (1 H, t, J = 7.6 Hz, H-7); ^{13}C NMR (CD_3OD, 100 MHz) δ 153.2 (s, C-3), 149.3 (s, C-5), 144.4 (d, C-2), 136.7 (s, C-4a), 132.6 (s, C-8a), 128.3 (d,C-7), 124.0 (d, C-8), 117.9 (d, C-4), 117.7 (d, C-6); HMBC correlations: C-3 to H-2; C-4 to H-2; C-4a to H-6; C-5 to H-4,

H-6, H-7; C-6 to H-7, H-8; C-8 to H-6; C-8a to H-7, H-8; HR-ESI-MS (negative mode) *m/z* 160.0400 $[M-H]^-$ (calcd 160.0399 for $C_9H_6NO_2$); HR-ESI-MS (positive mode) *m/z* 162.0550 $[M+H]^+$ (calcd 162.0555 for $C_9H_8NO_2$), 184.0368 $[M+Na]^+$ (calcd 184.0375 for $C_9H_7NO_2Na$).

Cytotoxicity Assay. The effects of compounds **1-3** on the growth of human cancer cell lines, HepG2, HT-29, and A549, were investigated as follows. Cells in the exponential phase were seeded in 96-well culture plates at the confluence of 1×10^4 cells/well, kept in 37 ˚C, 5% CO_2 incubator for 24 h. The cancer cell line was exposed to the test compound at five different concentrations for 72 h. Then, 100 μL of MTT (0.5 mg/mL in PBS) was added to each well, and the plates were incubated at 37 ˚C for another 4 h. After incubation, the culture medium was replaced with 150 μL of DMSO, and the plates were shaken for 3 min to dissolve the crystals, then the optical density values were read on the microplate reader (BioTek Epoch) at 570 nm. All tests and analyses were carried out in triplicate. DMSO and taxol were applied as the blank control and positive control, respectively.

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