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Two new ortho benzoquinones from *Uncaria rhynchophylla*

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[ABSTRACT] The present study was designed to determine the chemical constituents of the stems and hooks of *Uncaria rhynchophylla*. The chemical constituents were isolated and purified from CH_2Cl_2 fraction by chromatography. Their structures were elucidated by spectroscopic analyses. Their cytotoxicity was tested using MTT method. Two new ortho benzoquinones, 3-diethylamino-5-methoxy-1, 2-benzoquinone **(1)** and 3-ethylamino-5-methoxy-1, 2-benzoquinone **(2)**, together with a known compound isorhynchophyllic acid **(3)** were isolated from *U. rhynchophylla*. These compounds were evaluated for their cytotoxicity against cancer cells A549, HepG2 and A2780. Compounds **1** and **2** were new ortho benzoquinones and showed weak antiproliferative activities on A549, HepG2 and A2780 cells. Compound **3** significantly inhibited the proliferation of A549, HepG2 and A2780 cells with IC_{50} values being 5.8, 12.8 and 11.8 μmol·L^{-1}, respectively.

[KEY WORDS] *Uncaria rhynchophylla*; Ortho benzoquinones; Spectroscopic identification

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Introduction

Uncaria rhynchophylla (Rubiaceae) is woody climber plants that mainly distributed in Guangdong, Guangxi, Hubei, Hunan, Guizhou, Fujian and Jiangxi provinces of China. In the latest Chinese Pharmacopoeia, the stems and hooks of *U. rhynchophylla* are collected as "Gou-Teng" for the treatment of hyperpyrexia, epilepsy, preeclampsia [1]. The stems and hooks of this plant can treat various nervous disorders, ameliorating symptoms associated with epilepsy and Alzheimer disease [2-4]. The phytochemical studies of *U. rhynchophylla* resulted in various types of compounds, including indole alkaloids, triterpenes, flavonoids, and phenylpropanoids [5]. In our continuation of chemical investigation on the stems and hooks of this plant, two new compounds 3-diethylamino-5-methoxy-1, 2-benzoquinone **(1)** and 3-ethylamino-5-methoxy-1, 2- benzoquinone **(2)**, as well as a known compound isorhynchophyllic acid **(3)** [6] were isolated in the present study. Herein, we described the isolation and structure elucidation of these compounds and their cytotoxicity against three cancer cell lines: human lung cancer cell A549, human liver cancer cell HepG2, and human ovarian cancer cell A2780.

Results and Discussion

Compound **1** was obtained as red amorphous powder and showed molecular formula $C_{11}H_{15}NO_3$ with five degrees of unsaturation based on the pseudo-molecular peak at *m/z* 210.112 0 $[M + H]^+$ (Calcd. 210.112 5 for $C_{11}H_{16}NO_3$) in HR-ESI-MS. The IR absorptions at 1 637 and 1 618 cm^{-1} were ascribable to two carbonyl groups, respectively. The ^{1}H NMR spectrum showed two high-field ethyl groups signals at δ_H 1.24 (6H, d, J = 6.9 Hz, H-3') and 3.48 (4H, q, J = 6.9 Hz, H-2'), one methoxyl at δ_H 3.78 (3H, s, -OCH_3), and two olefinic proton at δ_H 5.66 (1H, s, H-4) and 5.57 (1H, s, H-6). The ^{13}C NMR (Table 1) gave nine signals including four olefinic carbons (δ_C 160.5, 149.7, 105.4, 101.2) and two carbonyl carbons (δ_C 185.0, 178.8). Combined with the 5 degrees of unsaturation, we suggested that compound **1** was an ortho benzoquinone, compared to compound

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These authors have no conflict of interest to declare.

Table 1 ^{1}H and ^{13}C NMR data for compounds 1 and 2 in $CDCl_3$ (*J* in Hz)

No.	1[a]		2[b]	
	δ_H	δ_C	δ_H	δ_C
1		185.0		183.2
2		178.8		179.4
3		149.7		147.6
4	5.66 (1H, s)	101.2	5.73 (1H, s)	103.3
5		160.5		162.7
$-OCH_3$	3.78 (3H, s)	56.2	3.82 (3H, s)	56.8
6	5.57 (1H, s)	105.4	5.38 (1H, s)	96.0
1'			5.86 (1H, br.s)	
2'	3.48 (4H, q, *J* = 6.9 Hz)	47.1	3.15 (2H, dq, *J* = 6.6, 7.2 Hz)	37.6
3'	1.24 (6H, d, *J* = 6.9 Hz)	12.5	1.28 (3H, d, *J* = 7.2 Hz)	13.6

[a] Recorded at 300 MHz (^{1}H NMR) and 75 MHz (^{13}C NMR).
[b] Recorded at 600 MHz (^{1}H NMR) and 150 MHz (^{13}C NMR).

4-methoxy-3, 6-diphenyl-3, 5-cyclohexadiene-1, 2-dione [7].

The HMBC correlations from methyl signal at δ_H 1.24 (H-3') to δ_C 47.1 (C-2'), and from methylene signal at δ_H 3.48 (H-2') to δ_C 47.1 (C-3') and 149.7(C-3) described the presence of diethylamino group. HMBC cross-peaks from one olefinic proton signal at δ_H 5.57 (H-4) to δ_C 149.7 (C-3), 160.5 (C-5) and 178.9 (C-2), and from the other olefinic proton at δ_H 5.66 (H-6) to δ_C 160.5 (C-5), 184.9 (C-1) and 178.9 (C-2) indicated the presence of ortho benzoquinone. Furthermore, the HMBC correlations from methoxyl at δ_H 3.78 ($-OCH_3$) to δ_C 160.5 (C-5), and from methylene at δ_H 3.48 (H-2') to δ_C 149.7 (C-3) showed the position of methoxyl group at C-5 and diethylamino group at C-3. Therefore, the structure of compound **1** was determined to be 3-diethylamino-5-methoxy-1, 2-benzoquinone.

Compound **2** was isolated as red amorphous powder. Its molecular formula was confirmed as $C_9H_{10}NO_3$ at *m/z* 182.081 7 $[M + H]^+$ (Calcd. 182.081 2 for $C_9H_{11}NO_3$) by HR-ESI-MS with five degrees of unsaturation. The IR absorptions at 3 415, 1 637, and 1 618 cm^{-1} exhibited the presence of N-H bond and two carbonyl groups, respectively. In the ^{1}H NMR spectrum of compound **2**, one high-field ethyl group signals at δ_H 1.28 (3H, d, *J* = 7.2 Hz, H-3'), 3.15 (2H, dq, *J* = 6.6, 7.2 Hz), one methoxyl signal at δ_H 3.82 (3H, s, $-OCH_3$), two olefinic proton signals at δ_H 5.73 (1H, s, H-4), 5.38 (1H, s, H-6) and one NH proton at δ_H 5.38 (1H, br s) were observed. The NMR data of compound 2 were similar to compound **1,** except the absence of an ethyl group. Instead, an NH proton was present at δ_H 5.38 (1H, br s), suggesting that an ethylamino group was attached to C-3. Combined with the data of ^{13}C NMR, HSQC and HMBC spectrum, the structure of compound **2** was determined to be 3-ethylamino-5-methoxy-1, 2-benzoquinone.

Compound **3** was identified as isorhynchophyllic acid (**3**) by comparison of its spectral data with those of the literature [6].

Compounds **1** and **2** showed weak antiproliferative activities on A549, HepG2 and A2780 cells at the concentration of 50–100 μmol·L^{-1}. Compound **3** significantly inhibited the proliferation of A549, HepG2 and A2780 cells with IC_{50} values being 5.8, 12.8 and 11.8 μmol·L^{-1}, respectively.

Table 2 Cytotoxic activities of compounds against three cancer cell lines

Compound	IC_{50} (μmol·L^{-1})		
	A549 (Lung)	HepG2 (Liver)	A2780 (Ovarian)
3-(diethylamino)-5-methoxy-1, 2-benzoquinone	50.2 ± 0.6	97.2 ± 1.0	84.6 ± 1.8
3-(ethylamino)-5-methoxy-1, 2-benzoquinone	94.8 ± 1.5	> 100.0	98.8 ± 1.2
isorhynchophyllic acid	5.8 ± 1.8	12.8 ± 1.6	11.8 ± 1.9
gambogic acid	2.7 ± 1.3	3.0 ± 1.1	2.9 ± 2.1

Gambogic acid, the positive control

Experimental

General experimental procedures

The NMR spectra were measured on Bruker Avance 300 MHz and 600 MHz (Bruker Co., Karlsruhe, Germany) using TMS as internal standard. ESI-MS and HR-ESI-MS were recorded on Agilent Technologies 6520 Accurate-Mass Q-TOF LC/MS spectrometer (Agilent Technologies, Palo Alto, USA). The IR spectra were measured on a Nicolet iS10 spectrophotometer (Thermo Fisher Scientific Inc., MA, USA)