Two Novel Alkaloids from the Stem of *Saprosma hainanense* and Their Cytotoxic Activities *in Vitro*

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Received October 9, 2010; accepted December 7, 2010; published online January 5, 2011

Two novel alkaloids, saprosmine A (1) and saprosmine B (2), were isolated from the stem of *Saprosma hainanense* MERR., along with five known alkaloids: marcanine A (3); cleistopholine (4); 4-methoxycarbonyl-5,10-benzogquinolinequinone (5); liriodenine (6); and quinoline (7). The chemical structures were established on the basis of extensive spectroscopic (IR, 1D-NMR, 2D-NMR, MS) data analyses and by comparison with spectroscopic data reported in the literature. Compounds 1 to 6 were evaluated for *in vitro* cytotoxic activities against the SPC-A-1 (human lung cancer), BEL-7402 (human hepatocellular carcinoma), SGC-7901 (human gastric cancer), and K-562 (human myelogenous leukaemia) cancer cell lines. Compounds 1 and 2 exhibited weak cytotoxic activities against K-562 cells. Compounds 3 and 5 showed cytotoxic activities against all four cancer cell lines.

Key words *Saprosma*; Rubiaceae; alkaloid; cytotoxic activity

Saprosma hainanense MERR. is a rubiaceous plant endemic to Hainan Island, P. R. China. The *Saprosma* genus (Rubiaceae) is represented by about 30 species of shrubs or small trees, which are mainly distributed in the tropical Asian region. In China, five species are found in Hainan and Yunnan. All parts of the plants in the genus are fetid when bruised. *Saprosma merrillii* is employed in a traditional Chinese medicine preparation to treat cancer in China.[1,2] Iridoid glucosides and anthraquinones were isolated from *Saprosma scortechinii*[3,4] and *Saprosma fragrans*.[5] A literature survey indicated that no chemical and pharmacological study was reported previously on *S. hainanense*.

Results and Discussion

Our preliminary biological screening of the alcohol extract of the stem of this plant showed that it exhibited cytotoxic activities against BEL-7402 cells which were localized in the chloroform fraction. Further chromatographic purification led to the isolation of two novel alkaloids, saprosmine A (**1**) and saprosmine B (**2**), along with five known alkaloids (**3**—**7**), which were identified as marcanine A (**3**),[6] cleistopholine (**4**),[7] 4-methoxycarbonyl-5,10-benzogquinolinequinone (**5**)[8] (Fig. 1), liriodenine (**6**),[9,10] quinoline (**7**),[11] respectively, by comparison with previously published data. This is the first report of all the compounds isolated from this genus. Compound **5** was the first obtained from natural resources. In this paper, we describe the isolation and structural elucidation of the new compounds, as well as the *in vitro* cytotoxic activities of compounds **1**—**6**.

Saprosmine A (**1**), yellow powder, gave a molecular-ion peak at *m/z* 285 in electrospray ionization-mass spectra (ESI-MS), consistent with the molecular formula $C_{16}H_{15}NO_4$ based on its high resolution (HR)-ESI-MS data (*m/z* 308.0894 $[M+Na]^+$). The IR spectrum of **1** revealed an absorption peak for the NH group at 3440 cm^{-1}, while strong absorption peaks at 273 and 380 nm were observed in the UV spectrum. The ^1H-NMR spectrum (Table 1) indicated a methyl group at δ 2.58 (3H, d, $J=0.8$ Hz) bonded to an aromatic ring; two methoxyl groups at δ 2.93 (6H, s); four AA′BB′ aromatic protons at δ 8.22 (1H, dd, $J=8.0$, 0.8 Hz), 7.87 (1H, dd, $J=8.0$, 0.8 Hz), 7.60 (1H, dt, $J=8.0$, 1.2 Hz), and 7.81 (1H, dt, $J=8.0$, 1.2 Hz); and an isolated aromatic hydrogen atom at δ 6.74 (1H, d, $J=1.2$ Hz) coupled with the methyl group at δ 2.58. The ^{13}C-NMR (Table 1) and heteronuclear single quantum coherence (HSQC) experiment showed one ketone group (δ 176.7), one quaternary carbon (δ 99.1), and 11 sp^2 carbons (δ128.8, 126.7, 130.7, 130.0, 127.1, 160.8, 120.1, 136.1, 141.1, 135.7, 151.8). Together with the heteronuclear multiple bond connectivity (HMBC) spectrum (Fig. 2), the 11 sp^2 carbons were assigned to an aromatic ring and a pyridine ring, respectively. The correlation peaks between the protons of the methyl group and C-4, C-4a, and C-10a suggested that the methyl group was bonded to C-4. The correlation peaks between protons of two methoxyl groups and C-5 suggested that the two methoxyl groups were both bonded to C-5. The ^1H- and ^{13}C-NMR data of **1** were closely related to those of **3**, suggesting that these two molecules are closely related. Differences in the ^1H- and

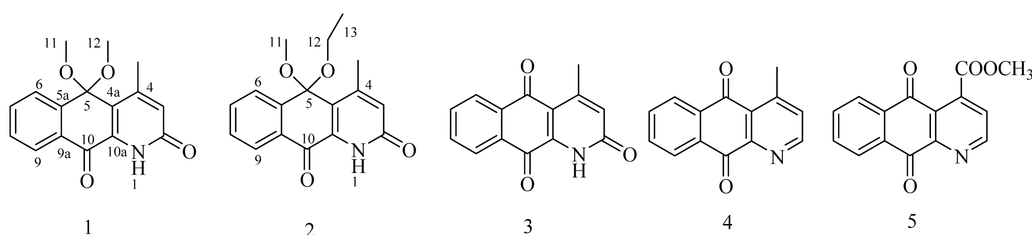

Fig. 1. The Structures of Compounds **1**—**5** Isolated from *S. hainanense*

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Table 1. [1]H- (400 MHz) and ^{13}C- (100 MHz) NMR Data of **1** and **2** ($CDCl_3$; δ in ppm, J in Hz)

	Compound **1**		Compound **2**	
	δ_C	δ_H	δ_C	δ_H
1				
2	160.8		160.8	
3	128.8	6.74 (1H, d, J=1.2 Hz)	128.8	6.72 (1H, d, J=0.8 Hz)
4	136.1		135.8	
4a	120.1		120.7	
5	176.7		176.7	
5a	141.1		141.8	
6	126.7	8.22 (1H, dd, J=8.0, 0.8 Hz)	126.7	8.19 (1H, dd, J=8.0, 0.8 Hz)
7	135.7	7.81 (1H, dt, J=7.2, 1.2 Hz)	135.8	7.80 (1H, dt, J=7.2, 1.2 Hz)
8	130.0	7.60 (1H, dt, J=8.0, 1.2 Hz)	130.0	7.58 (1H, dt, J=8.0, 1.2 Hz)
9	127.1	7.87 (1H, dd, J=8.0, 0.8 Hz)	127.1	7.88 (1H, dd, J=8.0, 0.8 Hz)
9a	130.7		130.5	
10	99.1		98.3	
10a	151.8		151.9	
11	51.4	2.93 (6H, s)	51.2	2.93 (3H, s)
12			59.6	2.90—3.12 (2H, m)
13			15.1	1.12 (3H, t, J=7.2 Hz)
Me	20.3	2.58(3H, d, J=0.8 Hz)	20.3	2.58(3H, d, J=0.8 Hz)
NH		9.74 (br s)		9.61 (br s)

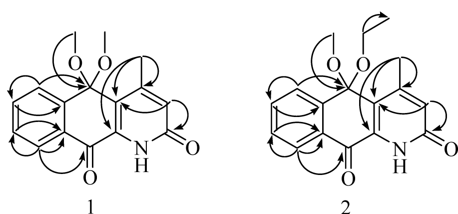

Fig. 2. Key HMBC Correlations of Compounds **1** and **2**

^{13}C-NMR spectra of **1** compared with those of **3** included the absence of two methoxyl groups at δ 2.93 (6H, s) and the quaternary carbon (δ 99.1), and the appearance of one ketone group. Additionally, the C-10a chemical shifts of **1** and **3** are almost identical (the C-10a chemical shift of **1** is δ 151.8, the C-10a chemical shift of **3** is δ 151.2), while the C-4a chemical shift of **1** is obviously different from that of **3** (the C-4a chemical shift of **1** is δ 120.1, the C-4a chemical shift of **3** is δ 115.1), suggesting that the quaternary carbon (δ 99.1) is bonded to C-4a. Therefore the structure of compound **1** was deduced to be saprosmine A (**1**), as shown in Fig. 1.

Saprosmine B (**2**), yellow powder, gave a molecular-ion peak at m/z 299 in ESI-MS, consistent with the molecular formula $C_{17}H_{17}NO_4$ based on its HR-ESI-MS data (m/z 322.1046 $[M+Na]^+$). The IR, UV, and NMR data of compound **2** were similar to those of **1**, suggesting that these two molecules are closely related. Differences in the ^{1}H-NMR spectrum of **2** compared with that of **1** included the absence of one methoxyl group at δ 2.93 and the appearance of an ethoxy group [δ_H 2.90—3.12 (2H, m), and 1.12 (3H, t, J=7.2 Hz)]. The differences in the ^{13}C-NMR spectra of **1** and **2** are similar to those observed in the ^{1}H-NMR spectra. Taken together, the NMR and MS evidence suggests that one methoxyl group in **1** is replaced by one ethoxy group in **2**. Therefore the structure of compound **2** was deduced to be saprosmine B (**2**), as shown in Fig. 1.

In HPLC, the chloroform extraction of the stem of *S. hainanense* also showed the same peak as compound **2**. Therefore we can conclude that compound **2** is not produced in EtOH extraction and is a new natural product.

Table 2. *In Vitro* Cytotoxic Activities ($IC_{50}/\mu M$) of Chloroform Fraction and Compounds **1** to **6** against Human Cancer Cell Lines

Compounds	IC_{50} (μM)			
	SPC-A-1	BEL-7402	SGC-7901	K-562
Chloroform fraction	37.9[a]	11.9[a]	18.9[a]	0.3[a]
1	N[b]	N	N	51.9
2	N	N	N	53.6
3	8.7	9.5	1.5	11.8
4	N	N	N	N
5	29.6	17.0	25.0	11.9
6	9.814[c]	71.7	33.7	197.7

a) Value in μg/ml. *b*) N means inactive at the tested concentration between 0.1—100 μM. *c*) Literature value[12] (in μg/ml).

Compounds **1**—**6** were evaluated for their cytotoxic activities against the SPC-A-1 (human lung cancer), BEL-7402 (human hepatocellular carcinoma), SGC-7901 (human gastric cancer), and K-562 (human myelogenous leukaemia) cancer cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay method as described by Skehan *et al.*[12] Compounds **3** and **5** showed cytotoxic activities against all four cancer cell lines, and the activities of compound **3** (with respective IC_{50} values of 8.7, 9.5, 1.5, 11.8 μM) were greater than those of compound **5** (with respective IC_{50} values of 29.6, 17.0, 25.0, 11.9 μM). Compounds **1** and **2** exhibited weakly cytotoxic activities against K562 cell, with IC_{50} values of 51.9 and 53.6 μM, respectively. Compound **6** was reported to exhibit cytotoxic activities against SPC-A-1 cells,[13] so we only tested the other cells. The IC_{50} values of compound **6** against the other three cells were 71.7, 33.7, and 197.7 μM, respectively. Compound **4** was inactive against all cell lines tested. The results are summarized in Table 2.

In summary, this study found evidence of anticancer activ-

ity of alkaloids from *S. hainanense*. Compounds **3** and **5** showed good cytotoxic activities. The activity of compound **3** is greater than that of the other six compounds, and therefore an investigation of the structural modification and structure-activity relationship of **3** will be considered in future. Furthermore, the chloroform fraction of *S. hainanense* showed good anticancer properties against K-562 cells, which may provide medicinal material in developing a potent anticancer Chinese patent medicine.

Experimental

General UV and IR spectra were recorded with PERSEE TU-1901 and Nicolet Avatar360 spectrometers, respectively. 1D- and 2D-NMR spectra were obtained on a Bruker DRX-400 with TMS as the internal standard. ESI-MS and HR-ESI-MS spectra were recorded on Finnigan-MAT-95-MS and Varian 7.0T FTICR-MS spectrometers, respectively.

Plant Material Stems of *S. hainanense* were collected in Bawangling county, Hainan province, P. R. China, in January 2008 and identified by Professor Qiong-xin Zhong (College of Life Science, Hainan Normal University). A voucher specimen (No. 080102) was deposited in the Key Laboratory of Tropical Medicinal Plant Chemistry of the Ministry of Education.

Extraction and Isolation Air-dried stems of *S. hainanense* (7 kg) were cut into small pieces and extracted with ethanol (75%), then fractionated to yield a petroleum ether extract (50 g), chloroform extract (105 g), ethyl acetate extract (95 g) and butanol extract (100 g). The chloroform extract was subjected to silica gel (200—300 mesh) column chromatography using a petroleum ether–acetone gradient (100 : 0 to 0 : 100) and finally washed with methanol to give 10 fractions. The fractions were combined on the basis of TLC comparison. Fr. 2 was further isolated by repeated silica gel chromatography with petroleum ether–acetone (50 : 1 to 1 : 1) and purified using Sephadex LH-20 chromatography and preparatory TLC (petrol ether–acetone, 2 : 1) to give compounds **1** (15 mg), **2** (10 mg), **4** (40 mg), and **5** (30 mg). Fr. 5 was chromatographed on silica gel with $CHCl_3$–CH_3OH (30 : 1 to 1 : 1) and further purified using preparatory TLC ($CHCl_3$–EtOAc, 3 : 1) to afford compounds **3** (150 mg), **6** (80 mg), and **7** (20 mg).

Cytotoxicity Assay In the MTT assay method, drug stock solutions were prepared in dimethyl sulfoxide (DMSO) and stored at -70 °C. Upon dilution in the culture medium, the final DMSO concentration was <1% DMSO (v/v). Cells were harvested at the exponential growth phase and seeded in flat bottomed 96-well microtiter plates. The cell volume in each well was 180 μl, containing 10^4 cells per well. The plates were incubated overnight in a 5% humidified CO_2 incubator at 37 °C. The compounds were then added to each well at various concentrations (100, 50, 10, 5, 1, 0.1 μM, respectively) using a constant volume of 20 μl, in sextuplicate, while maintaining a total well volume of 200 μl. After 48-h incubation at 37 °C in 5% CO_2, 50 μl of MTT (1 mg/ml of phosphate buffered saline (PBS)) was added to each well and again incubated at 37 °C for 4 h. After removing the medium carefully by aspiration, 150 μl of DMSO was added to each well and the formazan dye crystals were dissolved by shaking gently for 15 min. The plates were then read at 570-nm wavelength in an enzyme-labeled detector (Elx800, BioTek Instruments, Inc.). IC_{50} values of the compounds in different cell lines were determined based on the dose–response curve.

Analytical Data for Saprosmine A (**1**): 5,5-Dimethoxy-4-methylbenzo[*g*]quinoline-2,10(1*H*,5*H*)-dione: yellow powder. IR (KBr) cm^{-1}: 3440, 2925, 1731, 1656, 1612, 1463, 1397, 1330, 1289, 1224, 1074, 1023, 871, 804, 717, 682. UV λ_{max} (DMSO) nm (log ε): 273 (3.57), 380 (2.13). HR-ESI-MS *m/z*: 308.0894 ($[M+Na]^+$) (Calcd for $C_{16}H_{15}NO_4Na$ ($[M+Na]^+$), 308.0893). ESI-MS *m/z*: 286 ($[M+1]^+$), 571 ($[2M+1]^+$), 593 ($[2M+Na]^+$). ^{1}H- and ^{13}C-NMR data: see Tables 1 and 2.

Analytical Data for Saprosmine B (**2**): 5-Ethoxy-5-methoxy-4-methylbenzo[*g*]quinoline-2,10(1*H*,5*H*)-dione: yellow powder. IR (KBr) cm^{-1}: 3430, 2959, 2923, 2856, 1730, 1659, 1614, 1462, 1263, 1076, 1026, 804. UV λ_{max} (DMSO) nm (log ε): 273 (3.55), 380 (1.96). HR-ESI-MS *m/z*: 322.1046 ($[M+Na]^+$) (Calcd for $C_{17}H_{17}NO_4Na$ ($[M+Na]^+$), 322.1050). ESI-MS *m/z*: 300 ($[M+1]^+$), 599 ($[2M+1]^+$), 621 ($[2M+Na]^+$). ^{1}H- and ^{13}C-NMR data: see Tables 1 and 2.

Acknowledgment This work was financially supported by the Program for the National Natural Science Foundation of China (20862005) and Program for New Century Excellent Talents in University (NCET-08-0656).

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