Alkaloids from the Stem Bark of *Micromelum falcatum*

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Two new quinoldione alkaloids, methyl 2-(3-hydroxy-1-methyl-2,4-dioxo-1,2,3,4-tetrahydroquinolin-3-yl)-acetate (1) and 3-hydroxy-1-methyl-3-(2-oxopropyl)quinoline-2,4(1*H*,3*H*)-dione (2), and two quinolinone alkaloids previously synthesized but first isolated as natural products, *N*-methylflindersine (3) and 4-hydroxy-3-methoxy-1-methyl-2(1*H*)-quinolinone (4), were isolated from the stem bark of *Micromelum falcatum*, together with the known *N*-methylswietenidine-B (5). Their structures were established mainly on the basis of 1D- and 2D-NMR techniques. All compounds were evaluated for toxicity towards brine shrimp larvae, and 3 showed strong toxicity with an LD_{50} value of 1.39 μg/ml.

Key words *Micromelum falcatum*; alkaloid; quinoldione; quinolinone

Micromelum falcatum (LOUR.) TAN. (Rutaceae), traditionally used as Chinese folk medicine for curing infected wounds, odynolysis, and rheumatism, is widely distributed in Southeast Asia. The chemical constituents of *M. falcatum* had been previously investigated, and several coumarins, dihydrocinnamic acid derivatives, and two alkaloids 5,6-pyranoglycozoline and yuehchukene were obtained.[1,2] The dimeric indole alkaloid yuehchukene was found to have potent anti-implantation activity.[3] After further chemical investigation on the stem bark of *M. falcatum*, we report here the isolation of two new quinoldione alkaloids, methyl 2-(3-hydroxy-1-methyl-2,4-dioxo-1,2,3,4-tetrahydroquinolin-3-yl)acetate (**1**) and 3-hydroxy-1-methyl-3-(2-oxopropyl)-quinoline-2,4(1*H*,3*H*)-dione (**2**), two quinolinone alkaloids previously synthesized but first isolated as natural products, *N*-methylflindersine (**3**)[4,5] and 4-hydroxy-3-methoxy-1-methyl-2(1*H*)-quinolinone (**4**),[6] and the known *N*-methylswietenidine-B (**5**).[7] All isolates were tested for toxicity towards brine shrimp larvae.

Results and Discussion

The crude EtOH extract of the stem bark of *M. falcatum* was defatted with hexane and partitioned as described in the Experimental. The resulting AcOEt extract was subjected to silica gel column chromatography, sephadex LH-20, and semi-preparative HPLC to yield alkaloids **1**—**5**. Their structures were established by analysis of spectroscopic data.

Compound **1** was found to have the molecular formula $C_{13}H_{13}O_5N$ as determined by HR-electrospray ionization (ESI)-MS *m*/*z*: 264.0884 (Calcd for $C_{13}H_{14}O_5N^+$ $[M+H]^+$, 264.0872). Its UV spectrum showed absorption bands at λ_{max} 237 and 348 nm. Its IR spectrum revealed absorption bands at ν_{max} 1708 (carbonyl group) and 1667 cm^{-1} (an amide carbonyl group). The ^{1}H-NMR spectrum of **1** (Table 1) exhibited an ABCD aromatic system [δ_H 7.98 (dd, J=7.6, 1.4 Hz), 7.23 (dt, J=7.6, 1.4 Hz), 7.67 (dt, J=7.6, 1.4 Hz), 7.17 (dd, J=7.6, 1.4 Hz)], two methyls [δ_H 3.49 (s, N-CH_3), and 3.62 (s, OCH_3)], and one methylene [δ_H 2.96 (1H, d, J=14.6 Hz), 3.00 (1H, d, J=14.6 Hz)]. The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra of **1** showed the presence of two methyls [δ_C 30.5 (N–CH_3), and 52.3 (OCH_3)], one methylene (δ_C 43.8), four methines (δ_C 128.6, 123.9, 136.4, 115.2), and six quaternary carbons (δ_C 170.5, 78.4, 192.9, 120.4, 142.5, 169.1). These data were similar to those of haplotubinone[8] and 3,3-diisopentenyl-*N*-methyl-2,4-quinoldione,[9] and suggested that **1** also had a 1,2,3,4-tetrahydroquinoline-2,4-dione nucleus.

In the heteronuclear multiple-bond correlation (HMBC) spectrum, the correlations between δ_H 3.49 (s, N–CH_3) and δ_C 170.5 (C-2) and 142.5 (C-10), between δ_H 4.19 (s, OH) and δ_C 170.5 (C-2) and 192.9 (C-4) indicated the presence of a 2,4-quinoldione skeleton. Meanwhile, the HMBC correlations of the methylene proton δ_H 2.96, 3.00 (H-1′) with δ_C 169.1 (C-2′), 170.5 (C-2), 78.4 (C-3), and 192.9 (C-4), and the methyl proton δ_H 3.62 (s, OCH_3) with δ_C 169.1 (C-2′), revealed the structure of –CH_2COCH_3 located at C-3 of the 2,4-quinoldione skeleton. Based on these data, the structure of **1** was concluded to be methyl 2-(3-hydroxy-1-methyl-2,4-dioxo-1,2,3,4-tetrahydroquinolin-3-yl)acetate. Its rotation value was determined to be zero, which indicated that **1** was obtained as a racemate.

Compound **2** had the molecular formula $C_{13}H_{13}O_4N$ as established by HR-ESI-MS *m*/*z*: 248.0931 (Calcd for $C_{13}H_{14}O_4N^+$ $[M+H]^+$, 248.0923). The ^{1}H- and ^{13}C-NMR

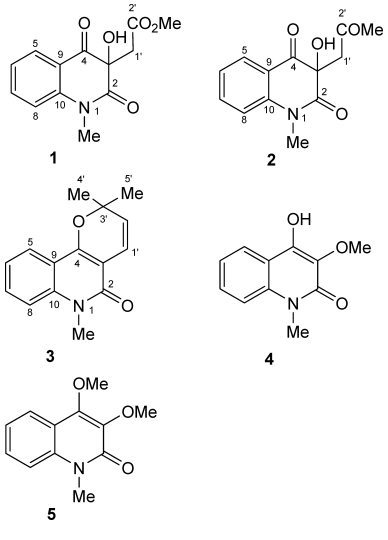

Fig. 1. Structures of Compounds **1**—**5**

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Table 1. ^{1}H- and ^{13}C-NMR Data[a] (500 and 125 MHz, Resp.; $CDCl_3$) for Compounds **1**—**3**

	1		2		3
	δ (C)	δ (H)	δ (C)	δ (H)	δ (C)
2	170.5		170.7		161.0
3	78.4		77.0		115.8
4	192.9		193.0		155.2
5	128.6	7.98 (dd, 7.6, 1.4 Hz)	128.3	7.89 (dd, 7.6, 1.6 Hz)	123.1
6	123.9	7.23 (dt, 7.6, 1.4 Hz)	123.4	7.13 (dt, 7.6, 1.6 Hz)	121.8
7	136.4	7.67 (dt, 7.6, 1.4 Hz)	136.2	7.58 (dt, 7.6, 1.6 Hz)	130.8
8	115.2	7.17 (dd, 7.6, 1.4 Hz)	115.1	7.12 (dd, 7.6, 1.6 Hz)	114.0
9	120.4		120.0		116.1
10	142.5		142.4		139.4
1′	43.8	2.96 (d, 14.6 Hz)	51.1	3.20 (d, 15.9 Hz)	118.0
		3.00 (d, 14.6 Hz)		3.25 (d, 15.9 Hz)	
2′	169.1		206.1		126.3
3′			30.7	2.10 (s)	78.7
4′					28.2
5′					28.2
H_3CN	30.5	3.49 (s)	30.1	3.38 (s)	29.3
H_3CO	52.3	3.62 (s)			
OH		4.19 (s)		4.60 (s)	

a) Assignments were accomplished using HSQC, HMBC and ^{1}H–^{1}H COSY experiments.

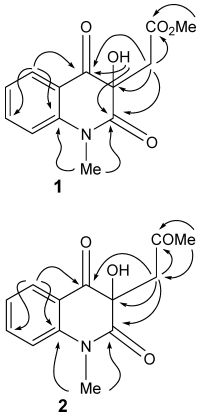

Fig. 2. Selected HMBC Correlations (→) of Compounds **1** and **2**

spectra of **2** (Table 1) were similar to those of **1** with the only difference being a carboxyl group [δ_C 169.1 (C-2′)] in **1**, substituted by a ketone group (δ_C 206.1) in **2**. This suggested that compound **2** was a 2,4-quinoldione alkaloid with an acetonyl group located at C-3, which was confirmed by the HMBC spectrum showing correlations of δ_H 3.20 (1H, d, J=15.9 Hz, H-1′) and 3.25 (1H, d, J=15.9 Hz, H-1′) with δ_C 206.1 (C-2′), 170.7 (C-2), 77.0 (C-3), and 193.0 (C-4), and of δ_H 2.10 (3H, s, H-3′) with δ_C 51.1 (C-1′) and 206.1 (C-2′). Thus, the structure of **2** was assigned to be 3-hydroxy-1-methyl-3-(2-oxopropyl)quinoline-2,4(1*H*,3*H*)-dione. The rotation value of **2** was determined to be zero, which was the same as **1**, indicating that compound **2** was also obtained as a racemate.

The toxicity of all compounds (**1**—**5**) was tested in the brine shrimp larvae assay, and their LD_{50} values were 143, 355, 1.39, 2020, and 70.5 μg/ml, respectively, indicating that compound **3** was a potent toxic natural product.

Experimental

General Experimental Methods Silica gel (200—300 mesh, Qingdao Haiyang Chemical Plant, Qingdao, China) and sephadex LH-20 (Pharmacia) were used for column chromatography. Thin layer chromatography (TLC) was carried out on precoated silica gel G plates (Qingdao Haiyang Chemical Plant) and spots were visualized by spraying the plates with 50% H_2SO_4 solution, followed by heating. NMR spectra were recorded on a Bruker DRX-500 (^{1}H-NMR, 500 MHz; ^{13}C-NMR, 125 MHz) spectrometer with $SiMe_4$ as an internal standard. ESI-MS were measured with an API2000 LC/MS/MS mass spectrometer (Applied Biosystems), and HR-ESI-MS were recorded using a VG Auto Spec-3000 MS spectrometer. Optical rotations and IR spectra were performed using a Polaptronic-HNQW5 high-resolution polarimeter and a Bruker VECTOR22 infrared spectrophotometer, respectively. A semipreparative Waters 600 HPLC system equipped with a Waters 996 photodiode array detector was carried out on octadecyl silica (ODS) columns (YMC-Pack ODS-5-A, 250×10 mm i.d., 5 μm, YMC) with the MeOH/H_2O solvent system.

Plant Material *M. falcatum* (Lour.) Tan. collected from Sanya, Hainan province, southern China, in October 2006, was authenticated by Prof. Si Zhang, South China Sea Institute of Oceanology, Chinese Academy of Sciences and a voucher specimen was deposited in the Herbarium of the South China Sea Institute of Oceanology (accession number: DAJIAN019).

Extraction and Isolation The air-dried material *M. falcatum* (Lour.) Tan. (10.0 kg) collected in Sanya, Hainan province, was extracted with 95% and 50% EtOH three times, respectively. After the organic solvent was evaporated under reduced pressure, the aqueous residue was subjected to extraction with *n*-hexane and EtOAc (three times, each). The EtOAc extract (113 g) was separated on silica gel (1600 g, 200—300 mesh) with solvents of increasing polarity: 10—70% acetone in *n*-hexane followed by 5—100% MeOH in $CHCl_3$ (165 frs). Fr. 5—11 (1.3 g, eluted with hexane–acetone 9 : 1) were chromatographed on silica gel using chloroform–acetone (50 : 1) and afforded 19.7 mg of **3**. Fr. 12—17 (0.29 g, eluted with hexane–acetone 4 : 1) were fractionated on silica gel with chloroform–acetone (20 : 1) and sephadex LH-20 with MeOH and yielded 15.4 mg of **5**. Fr. 39—43 (4.60 g, eluted with hexane–acetone 7 : 3) were fractionated on silica gel with chloroform–acetone (10 : 1) to yield 86.7 mg of **2** and 4.3 mg of **4**, then purified by semipreparative HPLC (250×10 mm i.d. 5 μm, MeOH/H_2O, 45 : 55) to yield 4.0 mg of **1**.

Methyl 2-(3-Hydroxy-1-methyl-2,4-dioxo-1,2,3,4-tetrahydroquinolin-3-yl)acetate (**1**): Colorless oil. $[\alpha]_D^{20}$ 0 (c=0.4, MeOH). UV λ_{max} (EtOH) nm (log ε): 348 (4.21), 237 (4.78). IR (KBr) cm $^{-1}$: 3428, 2952, 1708, 1667, 1604, 1474, 1362, 1210, 764. Positive ESI-MS *m/z*: 286 $[M+Na]^+$ (100), 264 $[M+H]^+$ (71), 232 $[M-OCH_3]^+$ (35), 214 (20), 189 (18). HR-ESI-MS *m/z*: 264.0884 (Calcd for $C_{13}H_{13}O_5N^+$ $[M+H]^+$, 264.0872). ^{1}H- and ^{13}C-NMR data: see Table 1.

3-Hydroxy-1-methyl-3-(2-oxopropyl)quinoline-2,4(1*H*,3*H*)-dione (**2**): Colorless oil. $[\alpha]_D^{20}$ 0 (c=1.5, MeOH). UV λ_{max} (EtOH) nm (log ε): 351 (4.09), 238 (4.92). IR (KBr) cm^{-1}: 3391, 2950, 1698, 1669, 1605, 1472, 1361, 1301, 1179, 762. Positive ESI-MS *m/z*: 517 $[2M+Na]^+$ (44), 270 $[M+Na]^+$ (100), 248 $[M+H]^+$ (93), 230 $[M-H_2O]^+$ (48), 202 (17). HR-ESI-MS *m/z*: 248.0931 (Calcd for $C_{13}H_{14}O_4N^+$ $[M+H]^+$, 248.0923). ^{1}H- and ^{13}C-NMR data: see Table 1.

N-methylflindersine (**3**): Colorless oil. IR, ^{1}H-NMR data: see ref. 5. ^{13}C-NMR data: see Table 1.

4-Hydroxy-3-methoxy-1-methyl-2(1*H*)-quinolinone (**4**): Crystals from CH_3OH. IR, ^{1}H- and ^{13}C-NMR data: see ref. 6.

N-methylswietenidine-B (**5**): Crystals from CH_2Cl_2. IR, ^{1}H- and ^{13}C-NMR data: see ref. 7.

Brine Shrimp Lethality Bioassay Following the reported method,[10,11] the brine shrimp lethality bioassay was carried out. Brine shrimp eggs (Ocean Star International, Inc., U.S.A.) were hatched in a large beaker having natural seawater (South China Sea), then incubated at room temperature for 48 h. With the help of a light source, the larvae were attracted to one side of the vessel and easily collected for the assay. Compounds **1**—**5** dissolved in dimethyl sulfoxide (DMSO) at a concentration of 50 mg/ml were diluted in 96-well plates with 200 μl seawater for testing at the final concentrations of 5, 50, and 500 μg/ml. Each test was conducted in triplicate with approximately 10 larvae, which were counted under a magnifying glass after 24 h incubation. The controls were prepared in the same manner except that the test samples were omitted. The lethality of larvae was recorded and used for calculating the LC_{50} with the Lanyu LC_{50} analysis program (ver. 1.01).

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