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(1H,3H)-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(1H)-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 LD50 value of 1.39 μg/ml.

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

Experimental

The resulting AcOEt extract was subjected to silica gel column chromatography, sephadex LH-20, and semi-preparative HPLC to yield alkaloids 1—5. 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 C13H13O5N as determined by HR-electrospray ionization (ESI)-MS m/z: 264.0884 (Calcd for C13H13O5N+ [M+H]+, 264.0872). Its UV spectrum showed absorption bands at \( \lambda_{\text{max}} \) 237 and 348 nm. Its IR spectrum revealed absorption bands at \( \nu_{\text{max}} \) 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 [\( \delta_\text{H} \) 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 [\( \delta_\text{H} \) 3.49 (s, N–CH3)], and 3.62 (s, OCH3)], and one methylene [\( \delta_\text{H} \) 2.96 (1H, d, J = 14.6 Hz), 3.00 (1H, d, J = 14.6 Hz)]. The \(^1^3\)C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra of 1 showed the presence of two methyls [\( \delta_\text{C} \) 30.5 (N–CH3)], and 52.3 (OCH3)], one methylene (\( \delta_\text{C} \) 43.8), four methines, and six quaternary carbons (\( \delta_\text{C} \) 170.5, 78.4, 192.9, 120.4, 142.5, 169.1). These data were similar to those of haplotubinone and 3,3-diisopentenyl-N-methyl-2,4-quinoldione, 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 \( \delta_\text{H} \) 3.49 (s, N–CH3) and \( \delta_\text{C} \) 170.5 (C-2) and 128.6, 123.9, 115.2, and 115.2, and six quaternary carbons (\( \delta_\text{C} \) 170.5, 78.4, 192.9, 120.4, 142.5, 169.1). These data were similar to those of haplotubinone and 3,3-diisopentenyl-N-methyl-2,4-quinoldione, and suggested that 1 also had a 1,2,3,4-tetrahydroquinoline-2,4-dione nucleus.

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Table 1. 1H- and 13C-NMR Dataa (500 and 125 MHz, Resp.; CDCl3) for Compounds 1—3

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a) Assignments were accomplished using HSQC, HMBC and 1H–1H 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 [δC 169.1 (C-2')] in 1, substituted by a ketone group (δC 206.1) in 2. This suggested that compound 2 was a 2,4-quinoldione alkaloid with an acetyl group located at C-3, which was confirmed by the HMBC spectrum showing correlations of δC 3.20 (1H, d, J=15.9 Hz, H-1') and 3.25 (1H, d, J=15.9 Hz, H-1') with δH 206.1 (C-2'), 170.7 (C-2), 77.0 (C-3), and 193.0 (C-4), and of δH 2.10 (3H, s, H-3') with δC 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(1H,3H)-dione.

The toxicity of all compounds (1—5) was tested in the brine shrimp larvae assay, and their LD50 values were 145, 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% H2SO4 solution, followed by heating. NMR spectra were recorded on a Bruker DRX-500 (1H-NMR, 500 MHz; 13C-NMR, 125 MHz) spectrometer with SiMe4 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 VICTOR22 infrared spectrophotometer, respectively. A semipreparative Waters 600 HPLC system equipped with a Waters 996 photodiode array detector was carried out on octadeyl silica (ODS) columns (YMC-Pack ODS-5-A, 250×10 mm i.d., 5 µm, YMC) with the MeOH/H2O 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% EtOH, and afforded 19.7 mg of 3.00 (d, 14.6 Hz), 3.25 (d, 15.9 Hz) and 206.1 (C-3) in 3. TAN. (10.0 kg) was separated on silica gel (1600 g, 200—300 mesh) with solvents 1: 348 (4.21), 237 (4.78). IR (KBr) cm−1: 3428, 2952, 1708, 1667, 1604, 1472, 1362, 1210, 764. Positive ESI-MS m/z: 286 [M+Na]+ (100), 264 [M+H]+ (71), 232 [M-OCH3]+ (35), 214 (20), 189 (18). HR-ESI-MS m/z: 264.0884 (Calcd for C13H14O3N+ [M+H]+, 264.0872). 1H- and 13C-NMR data: see Table 1.

-3-Hydroxy-1-methyl-3-(2-oxopropyl)quinoline-2,4(1H,3H)-dione (2): Colorless oil. [α]D0 0 (c=0.4, MeOH). UV λmax (EtOH) nm: 348 (4.21), 237 (4.78). IR (KBr) cm−1: 3428, 2952, 1708, 1667, 1604, 1472, 1362, 1210, 764. Positive ESI-MS m/z: 286 [M+Na]+ (44), 270 [M+Na]+ (100), 248 [M+H]+ (93), 230 [M−H]− (48), 202 (17). HR-ESI-MS m/z: 248.0931 (Calcd for C13H13O3N+ [M+H]+, 248.0923). 1H- and 13C-NMR data: see Table 1.

-N-methylfiddinesin (3): Colorless oil. IR, 1H-NMR data: see ref. 5. 13C-NMR data: see ref. 1.

-4-Hydroxy-3-methoxy-1-methyl-2(1H)-quinolinone (4): Crystals from CH3OH, IR, 1H- and 13C-NMR data: see ref. 6.

-N-methylsietiensidine-B (5): Crystals from CH3Cl, IR, 1H- and 13C-NMR data: see ref. 7.

Brine Shrimp Lethality Bioassay

Following the reported method,[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 LC50 with the Lanya LC50 analysis program (ver. 1.01).

Acknowledgments

The authors are grateful to the Knowledge Innovation Program of the Chinese Academy of Science (Grant KZCX2-YW-216),
and Hi-tech Research and Development Program of China (Grant SQ2007AA09Z409) for financial support.

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