Two Novel Alkaloids from *Zanthoxylum nitidum*

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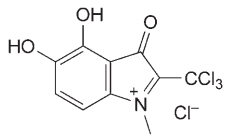
Two novel alkaloids, 4,5-dihydroxy-1-methyl-3-oxo-2-(trichloromethyl)-3*H*-indolium chloride (**1**) and 4-(2-methoxyethyl)-*N*,*N*-dimethylbenzenamine (**2**), were isolated from the EtOH extract of the roots of *Zanthoxylum nitidum*. Their structures were identified on the basis of spectroscopic and mass-spectrometric analyses.

Introduction. – In traditional Chinese medicine (TCM), the dried roots of *Zanthoxylum nitidum* (ROXB.) DC (Rutaceae), locally called '*liangmianzhen*', have been used for more than 1,000 years as an anti-inflammatory and analgesic agent. *Z. nitidum* is a morphologically variable species, found as a liane in rain forest, and as a shrub in dryer habitats. It is distributed from India to North Australia and Southeast China. In previous studies [1][2], some alkaloids, lignans, and flavones were isolated from this plant, whose constituents were found to exhibit significant bioactivities. For example, benzophenanthridine alkaloids from *Z. nitidum* inhibit the growth of *Ehrlich* ascites carcinoma cells [3], induce erythroleukemic cell differentiation by gene activation [4], and inhibit DNA topoisomerase-I [5].

Herein, we report two novel alkaloids from the EtOH extract of *Z. nitidum:* 4,5-dihydroxy-1-methyl-3-oxo-2-(trichloromethyl)-3*H*-indolium chloride (**1**) and 4-(2-methoxyethyl)-*N*,*N*-dimethylbenzenamine (**2**).



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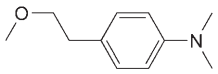

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Results and Discussion. – The BuOH-soluble fraction of the EtOH extract of *Z. nitidum* was purified by repeated column chromatography to afford compounds **1** and **2**, which were identified on the basis of their physical, mass-spectrometric, and spectroscopic properties, including 2D-NMR techniques such as HMQC, HMBC, and NOESY.

Compound **1** was obtained as yellow needles. HR-ESI-MS indicated the molecular formula $C_{10}H_7Cl_3NO_3$ (*m*/*z* 293.9496 (M^+; calc. 293.9492)). EI-MS showed four

isotopic peaks for M^+ at *m/z* 294 (42), 296 (40), 298 (13.5), and 299 (1.6) in a ratio of *ca.* 27:27:9:1, which pointed to three Cl-atoms in the molecule. The ^{13}C-NMR spectrum of **1** showed signals for 10 C-atoms: one MeN, one C=O, and one C=N group, an sp^3 C-atom, and six aromatic signals (two CH and four C). The aromatic region of the ^{1}H-NMR spectrum exhibited a pair of *doublet*s at δ(H) 7.27, 7.12 ($J = 9.0$ Hz), indicating two vicinal aromatic H-atoms. In the NOESY spectrum, the resonance at δ(H) 3.52 (*s*, 3 H) showed a cross-peak with δ(H) 7.27 (*d*), which suggested that the Me group was attached to the aromatic ring and in proximity to H–C(7), as confirmed by the HMBC spectrum (*Figure*). Also, the presence of a C=N group was established by HMBC analysis. Further, a C=O group was observed at δ(C) 180.1, which indicated a 3-oxo-3*H*-indolium skeleton. In addition, ^{13}C-NMR signals at δ(C) 151.5 (C(4)) and 134.9 (C(5)) indicated the connectivity with one OH group, respectively, while δ(C) 82.4 pointed to a Cl_3C function. Thus, from the above data, the structure of compound **1** was determined as 4,5-dihydroxy-1-methyl-3-oxo-2-(trichloromethyl)-3*H*-indolium chloride[1]).

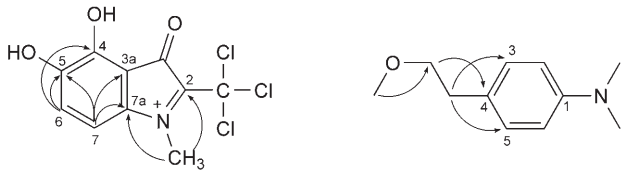

Figure. *Key HMBC* (H → C) *correlations for* **1** (left) *and* **2** (right)

Compound **2** was obtained as a colorless, amorphous solid. Positive HR-ESI-MS showed the $[M+H]^+$ signal at *m/z* 180.1385 (calc. 180.1388), in accord with the molecular formula $C_{11}H_{18}NO$. The ^{1}H- and ^{13}C-NMR spectra of **2** indicated one MeO, one Me_2N, an ethylene group [δ(H) 3.45, 3.01 ($J = 8.0$ Hz)], and a 1,4-disubstituted aromatic ring. The connectivity between the CH_2CH_2 moiety and the MeO function was established by an HMBC experiment (*Figure*). Thus, on the basis of these data, the structure of compound **2** was determined as 4-(2-methoxyethyl)-*N*,*N*-dimethylbenzenamine.

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Experimental Part

General. Column chromatography (CC): *Sephadex LH-20* (*Pharmacia*), *ODS* (25–40 μm; *Merck*), and *XAD-7 HP* gel (*Rohm & Haas*). Melting points (m.p.): *RY-2* apparatus (*Analytical Instruments Co.*, Tianjin, China); uncorrected. UV: *Shimadzu UV-265* apparatus; λ_{max} (log ε) in nm. IR: *Bruker Vector-22*

[1]) We cannot completely exclude that **1** is an artifact produced during extraction/purification, *e.g.*, by reaction of an indole derivative with $CHCl_3$ in the presence of acid or base. Compound **1** could not be detected by HPLC in the original EtOH extract.

spectrophotometer, with KBr pellets; in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *Bruker DRX-500* spectrometer, at 500 and 125 MHz, resp., in CD_3OD; δ in ppm rel. to Me_4Si, J in Hz. HR-ESI-MS: *Micromass Q-Tof* mass spectrometer; in m/z.

Plant Material. The roots of *Zanthoxylum nitidum* were collected in Guangxi Province, P. R. China, in August 2004, and identified by Prof. *Han-Chen Zheng*, Department of Pharmacognosy, School of Pharmacy, Second Military Medical University, Shanghai. A voucher specimen (No. 20040801) was deposited at the Herbarium of the School of Pharmacy, Second Military Medical University, Shanghai, P. R. China.

Extraction and Isolation. The air-dried, powdered roots (25 kg) of *Z. nitidum* were extracted with 80% aq. EtOH (30 l) at reflux. After removal of the EtOH under reduced pressure, the remaining brownish aq. syrup (6 l) was adjusted to pH 2 by addition of 2% aq. HCl, and then filtered. The filtrate was adjusted to pH 9 by adding 20% aq. NaOH soln., and then extracted with $CHCl_3$. The residue of the aq. phase was extracted with BuOH, and the resulting extract (125 g) was purified by CC (SiO_2; $CHCl_3$/MeOH gradient) to afford four fractions (*Fr. 1–4*). *Fr. 1* (2.2 g) was further purified by CC (*Sephadex LH-20*; MeOH) followed by recrystallization (MeOH) to yield **1** (48 mg). *Fr. 4* (7.4 g) was also purified by CC (*Sephadex LH-20*; MeOH) to afford a crude crystalline material (973 mg), which was further purified by CC (*ODS*; MeOH) to yield **2** (348 mg).

*4,5-Dihydroxy-1-methyl-3-oxo-2-(trichloromethyl)-3*H-*indolium Chloride* (**1**)[1]). Yellow needles. M.p. 164–176°. UV (MeOH): 225 (3.73), 285 (3.55), 300 (3.68). IR (KBr): 3395, 2520, 2247, 2073, 1718, 1578, 1487, 1464, 1435, 1421, 1321, 1280, 1220, 1182, 1142, 1079, 990, 896, 852, 829, 702, 649, 604. ^1H-NMR (500 MHz, $CDCl_3$): 7.27 (*d*, $J = 9.0$, H–C(7)); 7.12 (*d*, $J = 9.0$, H–C(6)); 3.52 (*s*, Me). ^{13}C-NMR (125 MHz, CD_3Cl): 180.1 (C(3)); 162.0 (C(2)); 151.5 (C(4)); 134.9 (C(5)); 122.8 (C(6)); 122.4 (C(3a)); 118.7 (C(7a)); 115.7 (C(7)); 82.4 (Cl_3C); 32.4 (Me). ESI-MS: 294.0 (M^+, $C_{10}H_7NO_3{}^{35}Cl_3^+$).

4-(2-Methoxyethyl)-N,N-*dimethylbenzenamine* (**2**). Colorless, amorphous solid. M.p. 91–105°. ^1H-NMR (500 MHz, CD_3OD): 7.18 (*d*, $J = 8.4$, H–C(2,6)); 6.86 (*d*, $J = 8.4$, H–C(3,5)); 3.45 (*t*, $J = 8.0$, $MeOCH_2$); 3.13 (*s*, MeO); 3.12 (*s*, Me_2N); 3.01 (*t*, $J = 8.0$, OCH_2CH_2). ^{13}C-NMR (500 MHz, CD_3OD): 154.2 (C(1)); 129.9 (C(3,5)); 127.1 (C(4)); 115.4 (C(2,6)); 66.8 ($MeOCH_2$); 52.6 (MeO); 52.6 (MeN); 27.6 ($MeOCH_2CH_2$). ESI-MS: 179.0 (M^+, $C_{11}H_{17}NO^+$).

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