Two New Benzophenanthridine Alkaloids from *Zanthoxylum nitidum*

by **Xin-Gang Cui**[a]), **Qing-Jie Zhao**[b]), **Qiao-Ling Chen**[b]), **Lei Xu**[c]), **Yan Song**[b]), **Yong-Sheng Jin***[b]), and **Dan-Feng Xu***[a])

[a]) Department of Urology, Changzheng Hospital, Second Military Medical University, Shanghai 200003, P. R. China (phone: +86-21-25070382; fax: +86-21-25070382; e-mail: smilewang@126.com, dfxu66@163.com)

[b]) Department of Organic Chemistry, School of Pharmacy, Second Military Medical University, Shanghai 200433, P. R. China

[c]) Pharmacy Department, Jiangsu Provincial Corps Hospital of Chinese People's Armed Police, Yangzhou City, Jiangsu Province, 225003, P. R. China

Two new benzophenanthridine alkaloids, named 8-methoxysanguinarine (**1**) and 8-methoxyisodecarine (**2**), were isolated from the EtOH extract of the rhizoma of *Zanthoxylum nitidum*. Their structures were determined on the basis of spectroscopic analysis.

Introduction. – *Zanthoxylum nitidum* (ROXB.) DC (Rutaceae) is a liane growing in rain forest or a shrub in dryer habitats. It is widely distributed throughout the southeastern part of China. Previous studies reported the isolation of alkaloids, lignans, and flavones [1–3]. Recent research on *Z. nitidum*, which has been used as anti-inflammatory and analgesic agent for more than 1000 years in traditional Chinese medicine, has shown several significant bioactivities. The benzophenanthridine alkaloids isolated from the title plant can inhibit the growth of *Ehrlich* ascites carcinoma cells [4], induce erythroleukemic cell differentiation by gene activation [5], and inhibit DNA topoisomerase I [6]. In our investigation on the anti-inflammatory and analgesic components from this plant, two new benzophenanthridine alkaloids, 8-methoxysanguinarine (**1**) and 8-methoxyisodecarine (**2**), were isolated from the $CHCl_3$ fraction of the EtOH extract of the rhizoma of *Z. nitidum*.

Results and Discussion. – The $CHCl_3$ fraction of the EtOH extract of *Z. nitidum* was purified by repeated column chromatography to afford compounds **1** and **2**. The two new alkaloids, 8-methoxysanguinarine (**1**) and 8-methoxyisodecarine (**2**), were identified as new compounds on the basis of spectroscopic analysis (*Fig.*)

Compound **1** was obtained as yellow powder. The HR-ESI-MS gave the molecular formula $C_{21}H_{16}NO_5^+$ (M^+ at m/z 362.3549; calc. 362.3555). The UV spectrum showed maxima at λ_{max} 235, 285, 324 (sh) nm (log ε 4.35, 4.58, 4.17), and minima at λ_{min} 255 nm (log ε 4.16), which indicated the benzophenanthridine moiety [7]. The ^{13}C-NMR spectrum showed 21 signals for C-atoms (1 MeN, 1 MeO, 2 OCH_2O, 6 CH (sp^2), and 11 C (sp^2); *Table*). The ^{1}H-NMR spectrum of **1** exhibited signals for six aromatic H-atoms (δ 8.49, 8.46, 8.07, 8.03, each *d*, $J = 9.0$ Hz; and 8.03 and 7.39, each *s*), one MeO (δ 3.85, *s*, 3 H), one MeN (δ 4.83, *s*, 3 H), and two OCH_2O units (δ 6.22 and 6.19, each *s*,

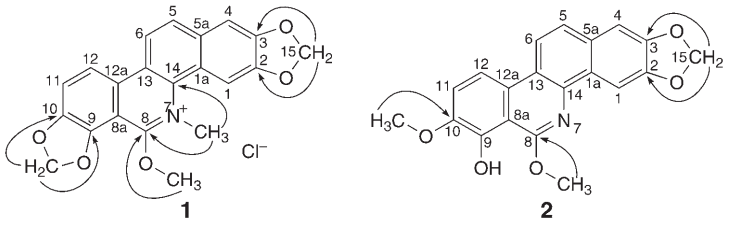

Figure. *The structures and key HMBC correlations* (H → C) *of* **1** *and* **2**[1])

Table. *[1]H- and [13]C-NMR Data of Compounds* **1** *and* **2**. [1]H-NMR at 500 MHz, [13]C-NMR at 125 MHz; in CD_3OD, δ in ppm, J in Hz.

Position[1])	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	8.03 (*s*)	104.7	8.55 (*s*)	101.4
C(1a)		121.2		120.0
C(2)		150.3		145.9
C(3)		150.4		147.4
H–C(4)	7.39 (*s*)	106.2	7.52 (*s*)	104.5
H–C(5)	8.07 (*d*, J = 9.0)	132.1	7.98 (*d*, J = 9.0)	127.1
C(5a)		133.9		129.1
H–C(6)	8.49 (*d*, J = 9.0)	117.9	8.53 (*d*, J = 9.0)	118.7
C(8)		163.4		162.7
C(8a)		129.2		126.4
C(9)		147.0		145.7
C(10)		151.1		148.1
H–C(11)	8.03 (*d*, J = 9.0)	127.0	7.60 (*d*, J = 9.0)	126.4
H–C(12)	8.46 (*d*, J = 9.0)	119.1	8.48 (*d*, J = 9.0)	118.6
C(12a)		120.2		118.1
C(13)		126.4		123.7
C(14)		132.7		128.0
CH_2(15)	6.19 (*s*)	103.4	6.22 (*s*)	101.8
MeN	4.83 (*s*)	50.1	–	–
MeO–C(8)	3.85 (*s*)	49.7	3.75 (*s*)	51.2
MeO–C(10)	–	–	3.93 (*s*)	59.9
9,10-OCH_2O	6.22 (*s*)	102.3	–	–

2 H). These data indicated a benzophenanthridine alkaloid [4]. The only ^{13}C-NMR difference between **1** and sanguinarine is that the CH_2 signal of sanguinarine was replaced by the signals for the segment C(8)–OMe (δ 163.4, 49.7), suggesting **1** to be a derivative of sanguinarine, which was further confirmed by the HMBC experiments (*Fig.*). Therefore, the structure of **1** was elucidated as 8-methoxysanguinarine[1]).

[1]) Arbitrary numbering. For systematic names, see *Exper. Part.*

Compound **2** exhibited a quasimolecular ion peak at *m/z* 372.3261 ($[M+Na]^+$) in the HR-ESI-MS, which corresponds to the molecular formula $C_{20}H_{15}NO_5$. The UV spectra showed maxima at λ_{max} 237, 287, 328 (sh) nm ($\log \varepsilon$ 4.40, 4.49, 3.92), and a minimum at λ_{min} 251 nm ($\log \varepsilon$ 3.99), which indicated the benzophenanthridine moiety [7]. Besides the two MeO substituents (δ 51.2 and 59.9), and one OCH_2O group (δ 101.8), the ^{13}C-NMR spectrum of **2** (*Table*) showed signals for 17 olefinic C-atoms (6 CH and 11 C), implying a benzophenanthridine alkaloid skeleton [7]. The OCH_2O group (CH_2(15)) was positioned at C(2) and C(3), and the two MeO groups at C(8) and C(10), respectively, based on the similarity of the NMR data of **1** and **2**, as well as HMBC correlations (*Fig.*) of the ^{1}H signal of the CH_2(15) group with C(1) and C(2), and of the two MeO signals (δ 3.75, 3.93) with C(8) and C(10), respectively. The NOESY spectrum of **2** showed correlations between H–C(11) and the ^{1}H signal of the MeO group (δ 3.93) at C(10). Consequently, the structure of compound **2** was unambiguously determined as 8-methoxyisodecarine[1]).

Experimental Part

General. Column chromatography (CC): *Sephadex LH-20* (*Pharmacia Fine Chemicals*, Piscataway, NJ, USA); *ODS* (25–40 μ; *Merck*); *XAD-7 HP* (*Rohm and Haas*, USA). M.p.: *RY-2* apparatus (*Analytical Instruments Co.*, Tianjin, China); uncorrected. IR: *Bruker Vector-22* spectrophotometer; KBr pellets, in cm^{-1}. NMR spectra: *Bruker DRX-500* spectrometer (500/125 MHz); in CD_3OD; δ in ppm rel. to Me_4Si, *J* in Hz. HR-ESI-MS: *Waters Q-TOF-micro-mass* spectrometer.

Plant Material. The rhizoma of *Zanthoxylum nitidum* were collected in Guangdong Province, China, in August of 2005 and identified by Prof. *Hang-Ming Zhang* of the Department of Pharmacognosy of this college. A voucher specimen (20050901) has been deposited at the Herbarium of School of Pharmacy, Second Military Medical University, Shanghai, P. R. China.

Extraction and Isolation. The air-dried and powdered rhizoma (5 kg) of *Z. nitidum* was refluxed with 75% EtOH (10 l). After removal of EtOH under reduced pressure, the aq. brownish syrup (2 l) was partitioned successively with petroleum ether, $CHCl_3$, and MeOH. Concentration of the solvents afforded a petroleum ether extract (312 g), a $CHCl_3$ extract (465 g), and a MeOH extract (512 g). The MeOH extract (200 g) was purified by CC (silica gel; gradient $CHCl_3$/MeOH) to afford six fractions (*Fr. A1–A6*). *Fr. A2* (3.2 g) was subjected to *ODS* CC to afford crude crystals (1.2 g), which were then purified by *Sephadex LH-20* CC (MeOH) to yield compounds **1** (47 mg) and **2** (43 mg).

8-Methoxysanguinarine (=*14-Methoxy-13-methyl[1,3]benzodioxolo[5,6-c]-1,3-dioxolo[4,5-i]phenanthridinium Chloride*; **1**). Yellow solid. M.p. 172–183°. UV: (CI^-) (EtOH): λ_{max} 235 (4.35), 285 (4.58), 324 (sh, 4.17), 343 (4.20), 355 (4.35), 419 (2.99), 508 (3.23); λ_{min} 255 (4.16), 315 (3.99), 347 (4.33), 407 (2.95), 448 (2.85). IR (CI^-): 3350, 1647, 1603, 1557, 1523, 1510, 1483, 1327, 1306, 1286, 1263, 1224, 1207, 1198, 1121, 1033, 1015, 980, 961, 937, 908, 875, 828, 790. ^{1}H- and ^{13}C-NMR: see the *Table.* EI-MS: 362 (2, M^+), 332 (100), 318 (12), 304 (6), 290 (18), 232 (4), 174 (12), 123 (6). ESI-MS: 362.4 (M^+). HR-EI-MS: 362.3549 (M^+, $C_{21}H_{16}NO_5^+$; calc. 362.3555).

8-Methoxyisodecarine (=*2,13-Dimethoxy[1,3]benzodioxolo[5,6-c]phenanthridin-1-ol*; **2**). Yellow solid. M.p. 203–214°. UV: (CI^-) (EtOH): λ_{max} 237 (4.40), 287 (4.49), 328 (sh, 4.35), 355 (3.92), 400 (3.07), 476 (3.12); λ_{min} 251 (3.99), 308 (4.11), 376 (2.97), 437 (2.92), 446 (1.06). IR: (CI^-) 3560, 3360, 1662, 1647, 1617, 1595, 1537, 1524, 1499, 1331, 1307, 1278, 1251, 1234, 1220, 1205, 1176, 1160, 1126, 1105, 1030, 1008, 978, 965, 915, 868, 843, 834. ^{1}H- and ^{13}C-NMR: see the *Table.* EI-MS: 349 (9, M^+), 318 (100), 189 (12), 174 (6), 123 (5), 84 (12), 57 (9), 44 (6). HR-EI-MS: 372.3261 ($[M+Na]^+$, $C_{20}H_{15}NO_5Na^+$; calc. 372.3266).

REFERENCES

[1] J. Hu, W.-D. Zhang, R.-H. Liu, C. Zhang, Y.-H. Shen, H.-L. Li, M.-J. Liang, X.-K. Xu, *Chem. Biodiv.* **2006**, *3*, 990; J. Hu, W.-D. Zhang, Y.-H. Shen, C. Zhang, L. Xu, R.-H. Liu, B. Wang, X.-K. Xu, *Biochem. Syst. Ecol.* **2007**, *35*, 114.
[2] M. M. Rahman, M. A. Islam, P. Khondkar, A. I. Gray, *Biochem. Syst. Ecol.* **2005**, *33*, 91.
[3] J. Hu, W.-D. Zhang, Y.-H. Shen, C. Zhang, R.-H. Liu, X.-K. Xu, B. Wang, *Helv. Chim. Acta* **2007**, *90*, 720.
[4] Z. X. Huang, Z. H. Li, *Acta Chim. Sin.* **1980**, *38*, 535.
[5] C. Dupont, E. Couillerot, R. Gillet, C. Caron, M. Zeches-Hanrot, J.-F. Riou, C. Trentesaux, *Planta Med.* **2005**, *71*, 489.
[6] S.-D. Fang, L.-K. Wang, S. M. Hecht, *J. Org. Chem.* **1993**, *58*, 5025.
[7] B. D. Krane, M. O. Fagbule, M. Shamma, B. Gözler, *J. Nat. Prod.* **1984**, *47*, 1.

Received September 19, 2007