Sinoraculine, the Precursor of the Novel Alkaloid Sinoracutine from *Stephania cepharantha* HAYATA

by **Li He**, **Lu-Lu Deng**, **Shu-Zhen Mu**, **Qian-Yun Sun**, **Xiao-Jiang Hao**, and **Yuan-Hu Zhang***

The Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences, Guiyang 550002, P. R. China (phone: +86-851-3806114; fax: +86-851-3805081; e-mail: zhangyuanhu@263.net)

A new alkaloid, sinoraculine, the precursor of the novel alkaloid sinoracutine, was isolated from the leaves and stems of *Stephania cepharantha*, and its structure was elucidated by spectroscopic analyses.

Introduction. – '*Jab fangx liangx*', the tuberous root of *Stephania cepharantha* HAYATA of the Menispermaceae family, a perennial herbaceous liana distributed widely in the northwest and southwest areas of China, is used as a folk medicine for the treatment of edema, gout, rheumatism, and arthralgia by the Miao minority in Guizhou Province of China [1]. Previously, we repored the isolation and structural determination of four novel alkaloids, cepharatines A–D, and some known ones from the stems and leaves of *S. cepharantha* HAYATA [2–4]. In further investigation of this plant, a new alkaloid, sinoraculine (*Fig. 1*) was isolated, which is the precursor of sinoracutine (**2**), an alkaloid with a novel skeleton isolated first from *Sinomenium acutum* (THUNB.) REHD. et WILS, Menispermaceae. Here, we describe the isolation and structural elucidation of sinoraculine.

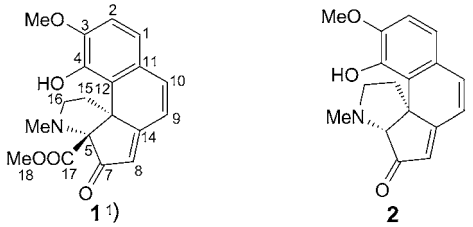

Fig. 1. *Structures of sinoraculine* (**1**) *and sinoracutine* (**2**)

Results and Discussion. – Sinoraculine (**1**), isolated as yellow crystals, had the molecular formula $C_{19}H_{19}NO_5$ according to its HR-ESI-MS (*m*/*z* 342.1342 ($[M+H]^+$; calc. 342.1341)), with eleven degrees of unsaturation. The UV absorptions recorded at 257 (0.658), 406 (0.430), and 413 nm (0.430) implied the presence of a highly conjugated system in **1**. The IR spectrum of **1** exhibited the absorption bands for OH (3451 cm^{-1}), ester function (1744 and 1256 cm^{-1}), conjugated C=O group (1691 cm^{-1}),

1) Arbitrary atom numbering. For systematics name, see the *Exper. Part*.

and aryl group (1603, 1550, 1452 cm^{-1}). The ^{13}C-NMR spectrum consisted of 19 signals corresponding to three Me (two oxygenated and one aminated), two saturated CH_2, five CH (two aromatic and three olefinic) groups, and nine quaternary C-atoms (two C=O, four aromatic, two saturated, and one olefinic).

The $^1H,^1H$-COSY and HMQC spectra revealed the presence of an isolated $-CH_2CH_2-$ fragment, and two pairs of vicinal H-atoms (*Fig. 2*). Further examination of the ^1H-, ^{13}C-, and 2D-NMR data, together with consideration of its degree of molecular unsaturation, suggested that compound **1** possesses a 6/6/5/5 tetracyclic skeleton with MeN, MeO, OH, and COOMe groups, and cyclopentanone moiety, which is similar to sinoracutine, a novel alkaloid first isolated from *S. acutum*. In the HMBC spectrum, correlation of MeN with C(16)[1]) revealed the presence of the $MeNCH_2CH_2$ group (*Fig. 2*), connected to C(5) and C(13) on the basis of the correlation of NMe with C(5), and H–C(15) with C(13). Correlations of H–C(8)/C(5) and H–C(8)/C(7) indicated that the C=O C-atom of the cyclopentenone unit was C(7). The C=C bond of this substructure was thus located between C(8) and C(14), a contention supported by the correlations H–C(8)/C(14) and H–C(9)/C(14). The MeO group was designated to C(3) by correlation of MeO–C(3)/C(3) and H–C(1)/C(3). The remaining COOMe group, which was deduced from the IR (1744, 1256 cm^{-1}) and NMR (δ(H) 3.43 (*s*); δ(C) 52.4 (*q*), 167.0 (*s*)) data and supported by correlation of H–C(18)/C(17), was thus at C(5). Therefore, the structure of **1** was established as shown in *Fig. 1*. Similar to sinoracutine, the pyrrolidine ring of **1** should be fused *syn* to the cyclopentenone unit at C(5) and C(13). The configuration of the quaternary C-atom C(5) is assumed to be (*R*) for biogenetic reasons. This deduction was further confirmed by its CD spectrum (229, +56.34; 284, −7.47; 412, −23.65 nm) [5][6].

Structurally, **1** is the precursor of sinoracutine, and the isolation of **1** lends valuable support for the proposed biosynthetic pathway of sinoracutine [5].

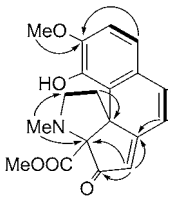

Fig. 2. *$^1H,^1H$-COSY* (—) *and key HMBC* (H → C) *correlations of* **1**

We thank the *Key Laboratory of Chemistry for Natural Products* of Guizhou Province and *Chinese Academy of Sciences*, the *Kunming Institute of Botany*, *Chinese Academy of Sciences*, and the *Institute of Materia Medica*, *Chinese Academy of Medical Sciences* for recording of the NMR, HR-ESI-MS, and CD data. This work was supported by grants from the *Science and Technology Commission of China* (No. 2009CB526512).

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 200–300 mesh and 10–40 μm; *Qindao Marine Chemical Ltd.*, Qindao, P. R. China), *RP-18* gel (50 μm; *YMC*, Japan), and *Sephadex LH-20* (40–70 μm; *Amersham Pharmacia Biotech AB*, S-Uppsala). TLC: Glass precoated with silica gel GF_{254}, and spots were visualized using *Dragendorff*'s reagent. M.p.: *X-4* melting-point apparatus. Optical

rotation: *Rudolph Autopol 1* digital polarimeter (2.5-cm cell). UV Spectra: *Shimadzu UV-2401* PC UV/VIS spectrophotometer; λ_{max} (log ε) in nm. CD Spectra: *JASCO-815* spectropolarimeter. IR Spectra: *Bruker Tensor 27* FT-IR spectrometer; KBr disks; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker DPX-500* NMR spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: *VG Auto Spec-3000* mass spectrometer; in *m*/*z*.

Plant Material. The stems and leaves of *Stephania cepharantha* HAYATA were collected in Guizhou Province, P. R. China, in June, 2008, and identified by Prof. *De-Yuan Chen* at Guiyang College of Traditional Chinese Medicine. A voucher specimen (No. Zhang20080616) has been deposited with the Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences.

Extraction and Isolation. Dried and powered stems and leaves (27 kg) of *S. cepharantha* were percolated with 95% EtOH at r.t. (3×5 d). After removal of solvent under reduced pressure, the residue was suspended in H_2O and extracted successively with petroleum ether and $CHCl_3$. The $CHCl_3$ extract (400 g) was chromatographed on SiO_2 column and eluted with $CHCl_3$/MeOH (from 100:0 to 0:100) to give eight major fractions, *Fr. 1* – *Fr. 8*. *Fr. 4* (4.0g) was subjected to CC (SiO_2; petroleum/acetone 8:2; and *RP-18*; MeOH/H_2O at 30, 50, 70%). The fraction eluted by 30% MeOH was further purified by CC (*Sephadex LH-20*; MeOH; and SiO_2; petroleum/acetone 8:2) to yield **1** (8 mg).

Sinoraculine (= *Methyl (3a*R*,11b*R*)-2,3-Dihydro-11-hydroxy-10-methoxy-3-methyl-4-oxo-1*H*-benzo[6,7]indeno[1,7a*-b*]pyrrole-3a(4*H*)-carboxylate*; **1**). Yellow crystals (MeOH). M.p. 136–138°. $[\alpha]_D^{15} = -14.54$ ($c = 0.80$, $CHCl_3$). UV ($CHCl_3$): 257 (0.658), 413 (0.430), 406 (0.429). CD (MeOH): λ_{ext} ($[\Delta\varepsilon]$) 229 (+56.34), 284 (−7.47), 412 (−23.65). IR (KBr): 3451, 1744, 1691, 1603, 1452, 1256, 1092. ^{1}H- and ^{13}C-NMR: *Table*. EI-MS: 341 (M^+), 323, 300, 282. HR-ESI-MS: 342.1342 ($[M+H]^+$).

Table. *NMR Data* ($CDCl_3$, 500 for ^{1}H and 125 MHz for ^{13}C) *of Sinoraculine* (**1**). δ in ppm, *J* in Hz. Atom numbering as indicated in *Fig. 1*.

Position	δ(H)	δ(C)	HMBC
H–C(1)	6.77 (*d*, *J* = 8.0)	121.8 (*d*)	C(2), C(3), C(4), C(9), C(10), C(11), C(12)
H–C(2)	6.72 (*d*, *J* = 8.0)	109.7 (*d*)	C(1), C(3), C(4), C(11)
C(3)		151.3 (*s*)	
C(4)		145.1 (*s*)	
C(5)		82.7 (*s*)	
C(7)		201.9 (*s*)	
H–C(8)	5.98 (*s*)	122.8 (*d*)	C(5), C(7), C(9), C(13), C(14), C(15)
H–C(9)	6.59 (*d*, *J* = 9.5)	117.5 (*d*)	C(11), C(12), C(14)
H–C(10)	6.82 (*d*, *J* = 9.5)	138.7 (*d*)	C(1), C(4), C(7), C(12), C(13), C(14)
C(11)		124.0 (*s*)	
C(12)		126.3 (*s*)	
C(13)		59.2 (*s*)	
C(14)		175.9 (*s*)	
CH_2(15)	2.23–2.25 (*m*, H_α), 2.16–2.20 (*m*, H_β)	40.3 (*t*)	C(5), C(12), C(13), C(14), C(16)
CH_2(16)	3.27–3.31 (*m*, H_α), 2.88–2.91 (*m*, H_β)	52.2 (*t*)	C(5), C(13), C(15), MeN
C(17)		167.0 (*s*)	
H–C(18)	3.43 (*s*)	52.4 (*q*)	C(17)
*Me*O–C(3)	3.87 (*s*)	55.7 (*q*)	C(3)
MeN	3.03 (*s*)	36.3 (*q*)	C(5), C(16)

REFERENCES

[1] Editorial Committee of Chinese Materia Medica under the State Administration of Traditional Chinese Medicine, 'Chinese Materia Medica, Vol. of Traditional Miao Medicine', Guizhou Science and Technology Press, Guiyang, Guizhou Province, 2005, p. 71.
[2] L. He, Y.-H. Zhang, L.-J. Tang, S.-H. Song, Q.-Y. Sun, *China J. Chin. Mater. Med*. **2010**, *35*, 1272.
[3] L. He, Y.-H. Zhang, L.-J. Tang, S.-H. Song, Q.-Y. Sun, *J. Chin. Med. Mater*. **2010**, *33*, 1568.
[4] L. He, Y.-H. Zhang, H.-Y. Guan, J.-X. Zhang, Q.-Y. Sun, X.-J. Hao, *J. Nat. Prod*. **2011**, *74*, 181.
[5] G.-H. Bao, X.-L. Wang, X.-C. Tang, C. Pauline, G.-W. Qin, *Tetrahedron Lett*. **2009**, *50*, 4375.
[6] V. I. Minkin, M. Legrand, M. J. Rougier, 'Determination of Configurations by Dipole Moments, CD or ORD', Georg Thieme Publishers, 1977.

Received January 6, 2012