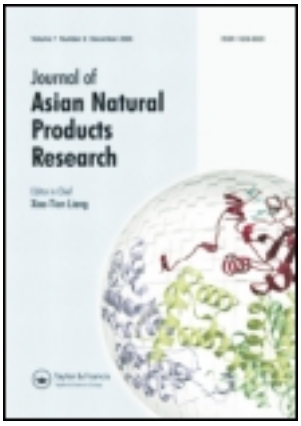

Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

Two new morphinane alkaloids from *Sinomenium acutum*

Xiao-Ling Wang [a], Bing-Rui Liu [a b], Jun-Ru Wang [b], Chien-Kuang Chen [d], Guo-Wei Qin [c] & Shoei-Sheng Lee [d]

[a] Key Laboratory of Phytochemistry of Shaanxi Province, Baoji University of Arts and Sciences, Baoji, 721013, China

[b] College of Science, Northwest Agriculture and Forest University, Yangling, 712100, China

[c] Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, 201203, China

[d] School of Pharmacy, College of Medicine, National Taiwan University, Taipei, 100, Taiwan, China

Available online: 25 May 2011

To cite this article: Xiao-Ling Wang, Bing-Rui Liu, Jun-Ru Wang, Chien-Kuang Chen, Guo-Wei Qin & Shoei-Sheng Lee (2011): Two new morphinane alkaloids from *Sinomenium acutum*, Journal of Asian Natural Products Research, 13:06, 523-528

To link to this article: http://dx.doi.org/10.1080/10286020.2011.574617

Journal of Asian Natural Products Research
Vol. 13, No. 6, June 2011, 523–528

Two new morphinane alkaloids from *Sinomenium acutum*

Xiao-Ling Wang[a]*, Bing-Rui Liu[ab], Jun-Ru Wang[b], Chien-Kuang Chen[d], Guo-Wei Qin[c] and Shoei-Sheng Lee[d]

[a]*Key Laboratory of Phytochemistry of Shaanxi Province, Baoji University of Arts and Sciences, Baoji 721013, China;* [b]*College of Science, Northwest Agriculture and Forest University, Yangling 712100, China;* [c]*Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 201203, China;* [d]*School of Pharmacy, College of Medicine, National Taiwan University, Taipei 100, Taiwan, China*

(*Received 17 January 2011; final version received 20 March 2011*)

Two new morphinane alkaloids, 1-hydroxy-10-oxo-sinomenine (**1**) and 4,5-epoxy-14-hydroxy sinomenine N-oxide (**2**), have been isolated from the stems of *Sinomenium acutum*. Their structures were established by various spectral analyses, especially 2D NMR experiments. The structure of **2** was confirmed by single crystal X-ray diffraction. The absolute configurations of **1** and **2** were deduced by comparison of CD spectra with the known alkaloid sinomenine (**3**). Compound **1** was tested for DPPH inhibition and gave IC_{50} of 27.9 μM. Compound **2** was tested for neuroprotective effect and showed significant activity against β-amyloid$_{25-35}$-induced oxidative injury ($^*P < 0.05$) at 10 μM in PC-12 cells.

Keywords: *Sinomenium acutum*; 1-hydroxy-10-oxo-sinomenine; 4,5-epoxy-14-hydroxy sinomenine N-oxide

1. Introduction

Sinomenium acutum (Menispermaceae) is a deciduous twining vine distributed widely in the hilly regions of southwest, northwest, and southeast China [1]. The plant stems have been identified in Chinese Pharmacopoeia as a traditional Chinese medicine for the treatment of rhematalgia, rheumatism, and arthralgia [2]. It is known that *S. acutum* contains rich alkaloids with various structure skeletons. Sinomenine, a known morphinane-type alkaloid, is the main component of the plant and showed inhibition to inflammatory reaction and lymphocyte proliferation, so as to be used as an antiarthritic drug in clinic [3]. In our previous study, two new morphinane alkaloid dimers [4], a new hausbanane-type alkaloid [5] and a new skeleton alkaloid sinoracutine [6], were isolated from the plant stems. In order to search other components of the plant, a remained residue after benzene extraction for sinomenine was investigated, resulting in the isolation of two new minor alkaloids. Here, we report the structures of two new morphinane alkaloids 1-hydroxy-10-oxo-sinomenine (**1**) and 4,5-epoxy-14-hydroxy sinomenine N-oxide (**2**) (Figure 1), as well as the antioxidant activity of **1** on DPPH radical scavenging assay and neuroprotective effect of **2** against hydrogen peroxide and β-amyloid$_{25-35}$-induced oxidative injury.

2. Results and discussion

Alkaloid **1** was obtained as a white amorphous powder. The molecular formula

*Corresponding author. Email: xlwang6811@hotmail.com

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020.2011.574617
http://www.informaworld.com

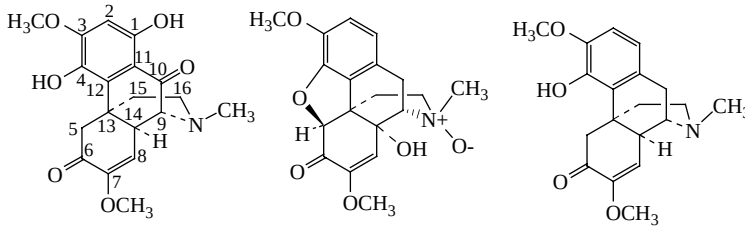

Figure 1. The structures of alkaloids **1**–**3**.

was determined to be $C_{19}H_{21}NO_6$ by ^1H and ^{13}C NMR spectral data (see Table 1) and the pseudo-molecular ion in HR-ESI-MS at *m/z* 382.1261 $[M + Na]^+$. The UV absorption maxima recorded at 248 (4.20), 272 (4.08), and 294 (3.90) nm revealed that **1** possessed conjugated systems. The IR spectrum showed the characteristic absorption bands of hydroxyl (3304 cm^{-1}) and α,β-unsaturated carbonyl (1688, 1618 cm^{-1}) groups. The ^{13}C NMR spectrum showed 19 signals for three oxygenated and/or nitrogenated methyl, three methylene, four methine (one aromatic, one olefinic), and nine quaternary (two carbonyl, six aromatic, and/or olefinic) carbons. The ^1H NMR spectrum showed the signals for two methoxyls (δ 3.85, 3.44, each 3H, s), one N-methyl (δ 2.38, 3H, s), one aromatic proton (δ 6.29, 1H, s) and one olefinic proton (δ 5.33, 1H, d, $J = 2.0$ Hz). It also displayed a much downfield signal at δ 12.95 as a singlet, which disappeared after the addition of D_2O, indicating the

Table 1. The NMR spectral data of alkaloids **1** and **2** ($CDCl_3$; δ in ppm and J in Hz).

	(1)		(2)	
Pos.	^1H NMR	^{13}C NMR	^1H NMR	^{13}C NMR
1		159.2(s)	6.62 (d, $J = 8.0$)	120.1(d)
2	6.29(s)	98.2(d)	6.72 (d, $J = 8.0$)	116.1(d)
3		155.0(s)		143.8(s)
4		137.2(s)		144.2(s)
5	2.42(d, $J = 15.8$, H-5α) 4.32(d, $J = 15.8$, H-5β)	49.0(t)	4.94(s)	87.1(d)
6		192.6(s)		188.7(s)
7		152.9(s)		154.6(s)
8	5.33(d, $J = 2.0$)	112.9(d)	5.65(s)	114.6(d)
9	3.32(d, $J = 2.0$)	66.8(d)	3.77(d, $J = 5.2$)	75.0(d)
10		197.8(s)	3.13(dd, $J = 19.2$, 5.2, H-10β) 3.35(d, $J = 19.2$, H-10α)	29.0(t)
11		113.1(s)		119.8(s)
12		123.6(s)		130.6(s)
13		46.6(s)		46.6(s)
14	3.12(t, $J = 2.0$)	45.9(s)		70.7(s)
15	1.93(1H, br d, $J = 12.9$, H-15β) 2.04(1H, br d, $J = 12.9$, H-15α)	35.2(t)	3.26–3.22(m, H-15β) 1.82(d, $J = 14.5$, H-15α)	24.8(t)
16	2.11(br d, $J = 12.0$, H-16α) 2.69(dd, $J = 12.0$, 3.5, H-16β)	47.1(t)	3.26–3.22(m, H-16α) 3.33(m, H-16β)	61.9(t)
3-OCH_3	3.85(s)	56.2(q)	3.95(s)	56.9(q)
7-OCH_3	3.44(s)	54.9(q)	3.51(s)	55.2(q)
N-CH_3	2.38(s)	43.2(q)	3.35(s)	59.5(q)
1-OH	12.95(s)			

presence of a carbonyl-chelated hydroxyl group. Further studies showed that the ^{1}H and ^{13}C NMR spectral data of **1** (Table 1) were very similar to those of sinomenine **3** [7], except two differences: (1) Two ortho-coupled aromatic protons (δ 6.56, 6.68, each 1H, d, $J = 8.2$ Hz, H-1 and 2) in sinomenine **(3)** were changed to an isolated proton (δ 6.29, 1H, s, H-2) in **1**. Meanwhile, an isolated aromatic methine was changed as an oxygenated quaternary carbon at δ_C 159.2 in **1**. (2) The signal of C-10 methylene in sinomenine **(3)** was substituted by a carbonyl (δ_C 197.8) in **1**. The above evidence suggested that **1** has the additional C-1 hydroxyl and C-10 carbonyl groups compared with **3**. The HMBC correlations of hydroxyl at C-1 with C-2 and C-11; H-14 with C-10 (Figure 2) confirmed the proposed structure of **1**. The NOESY cross-peaks of H-14/H-5α, H-5α/H-15α and H-15α/H-14, H-14/N-CH_3 (Figure 2) indicated that H-14 and N-containing ring were on the same side with α-orientation [4]. The absolute configuration of **1** was the same with that of sinomenine **3**, deduced by CD spectrum of positive ($\Delta\varepsilon$, +38.39) at 247 nm and negative ($\Delta\varepsilon$, −22.62) at 301 nm, which was similar to those of sinomenine [6]. Thus, the new alkaloid **1** was elucidated as 1-hydroxy-10-oxo-sinomenine.

Alkaloid **2** was obtained as colorless block crystals. The molecular formula was determined to be $C_{19}H_{21}NO_6$ by ^{1}H and ^{13}C NMR spectral data (see Table 1), and the pseudo-molecular ion in HR-ESI-MS at *m/z* 382.1261 $[M + Na]^+$. The UV absorption maxima recorded at 231 (4.05), 259 (3.77), and 285 (3.62) nm revealed that **2** possessed conjugated system. The IR spectrum showed the characteristic absorption bands of hydroxyl (3441 cm^{-1}), α,β-unsaturated carbonyl (1693, 1621 cm^{-1}), and C—O—C (1281 and 1164 cm^{-1}) groups. The ^{13}C NMR spectrum showed 19 signals for three oxygenated and/or nitrogenated methyl, three methylene, five methine (two aromatic, one olefinic), and eight quaternary (one carbonyl, five aromatic, and/or olefinic) carbons. The ^{1}H NMR and HSQC experiments revealed one pair of aromatic ortho-pattern protons (δ 6.62, 6.72, each 1H, d, $J = 8.0$ Hz), one isolated olefinic proton (δ 5.65, 1H, s), three methyl (two methoxyls at δ 3.95 and δ 3.51 and one N-methyl at δ 3.35), an isolated —CH_2CH_2— fragment, and an isolated —CH_2CH— fragment. The NMR spectral feature was very similar to those of sinomenine N-oxide [7], except two differences: (1) C-5 methylene was changed as an oxygenated methine (δ_H 4.94, 1H, s; δ_C 87.1, d) and (2) C-14 methine was changed to an oxygenated quaternary carbon (δ_C 70.7), suggesting the presence of 4,5-expoxy and additional C-14 hydroxyl groups in **2**. This deduction was supported by strong correlations of H-5 with C-4 and H-9 with C-14 in HMBC spectrum (Figure 2). Finally, the proposed structure and its relative configuration were unambiguously confirmed by X-ray crystallographic analysis (Figure 3). The absolute

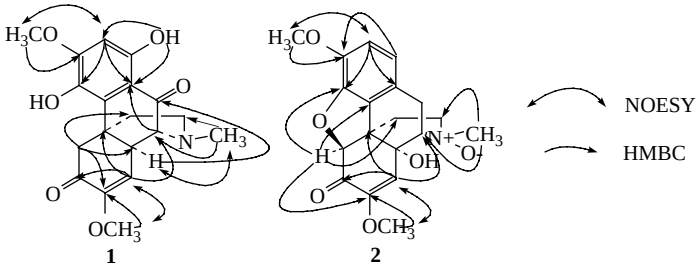

Figure 2. The key HMBC and NOESY correlations of compounds **1** and **2**.

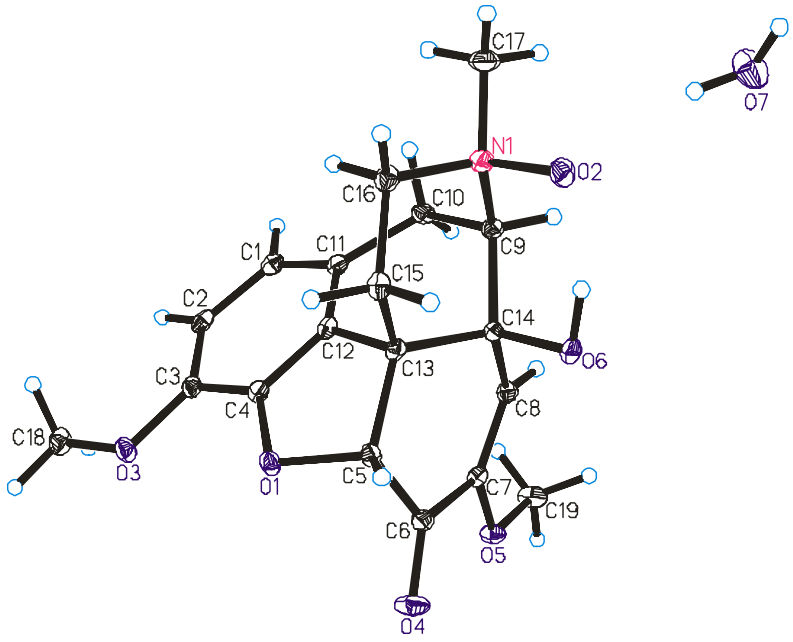

Figure 3. X-ray crystal structure of compound **2**.

configuration of **2** was the same with that of sinomenine, deduced by CD spectrum of positive ($\Delta\varepsilon$, +5.33) at 236 nm and negative ($\Delta\varepsilon$, −21.13) at 279 nm, which was similar to those of sinomenine **3**. Thus, the new alkaloid **2** was elucidated as 4,5-epoxy-14-hydroxy sinomenine N-oxide.

Alkaloids **1** and **2** were morphinane-type minor alkaloids. The presence of 10-oxo in **1** and 4,5-epoxy and 14-hydroxy groups in **2** were first reported in the sinomenine related alkaloids. Compound **1** was tested for DPPH inhibition and gave the IC_{50} of 27.9 μM. Compound **2** was tested for neuroprotective effect against hydrogen peroxide and β-amyloid$_{25-35}$-induced oxidative injury in PC-12 cells. It showed significant activity against β-amyloid$_{25-35}$-induced injury at a concentration of 10 μM ($^{*}P < 0.05$).

3. Experimental

3.1 General experimental procedure

Optical rotation was obtained with a JASCO DIP-370 polarimeter. IR spectra (KBr) were obtained with a JASCO FT/IR-410 spectrometer. UV spectra were recorded on a Hitachi U-2001 spectrophotometer. CD spectra were measured on a JASCO J-720 spectrometer in MeOH. HR-ESI-MS was obtained on a Finnigan MAT TSQ 7000 spectrometer. ^{1}H, ^{13}C, and 2D NMR data were determined on a Bruker AVANCE 500 and a Bruker AVANCE 600 spectrometers in $CDCl_3$. The chemical shifts were referenced to the residual solvent peak of $CDCl_3$, respectively. All solvents used were of analytical grade. Silica gel (200–300 and 300–400 mesh) was used for column chromatography, and precoated silica gel GF254 plates used for TLC (Qingdao Haiyang Chemical Company Ltd., Qingdao, China). X-ray diffraction was performed on a Siemens-P4 four-circle diffractometer equipped with a graphite-monochromatic MoK_α radiation ($\lambda = 0.71073$ Å) using an ω scan mode at 289(2) K. DPPH inhibition test was determined on a Bio Tek Powerwave XS2 automated microplate.

3.2 Plant material

The stems of *S. acutum* were collected from Baoji city of Shaanxi province and identified by Dr Zhijun Fu of Baoji University of Arts and Sciences. A voucher specimen (QT-20051024) has been deposited in the herbarium of the college.

3.3 Extraction and isolation

The powder of the plant stems was soaked and alkalized with 10% $Ca(OH)_2$ solution and then extracted with benzene. The benzene extracts were concentrated and deposited overnight to precipitate major alkaloid sinomenine. After removing of crude sinomenine, the remaining mother liquor was concentrated to provide a sticky residue.

The sticky residue (3 kg) was fractionated by column chromatography on silica gel (100 mesh) eluted with $CHCl_3$ to $CHCl_3$/MeOH (30:1; 10:1 and 4:1), gradually to afford seven fractions, QT1–QT7. The QT3 (600 g) was subjected to column chromatography on silica gel (200–300 mesh) eluted with petroleum ether/acetone (10:1 to acetone) to give 12 subfractions, QT3-1–QT3-12. The QT3-9 (10 g) was chromatographed repeatedly on silica gel (300–400 mesh) eluted with $CHCl_3$/MeOH (100:1 to 30:1) to yield **1** (14 mg). The QT3-10 (22 g) was chromatographed repeatedly on silica gel (300–400 mesh) eluted with $CHCl_3$/MeOH (100:1 to 10:1) to yield **2** (10 mg).

3.3.1 1-Hydroxy-10-oxo-sinomenone (**1**)

$[\alpha]_D^{23}$ −159.6 (*c* 0.307, MeOH); UV(MeOH) λ_{max} 248 (4.20), 272 (4.08), 294 (3.90) and 381 (3.94) nm; IR ν_{max} (cm^{-1}): 3304(OH), 1688, 1618 (C = C—C = O), 1479, 1437; ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectral data in $CDCl_3$, see Table 1; HR-ESI-MS: *m/z* 382.1261 $[M + Na]^+$ (calcd for $C_{19}H_{21}NO_6Na$, 382.1267); CD (MeOH) $\Delta\varepsilon_{247}$, +38.39, $\Delta\varepsilon_{301}$, −22.62.

3.3.2 4,5-Epoxy-14-hydroxy sinomenine N-oxide (**2**)

$[\alpha]_D^{23}$ 30.7 (*c* 0.228, MeOH); UV(MeOH) λ_{max} 231 (4.08), 259 (3.77), 285 (3.62) nm; IR ν_{max} (cm^{-1}): 3441(OH), 1693, 1620 (C═C—C═O), 1504 (phenyl), 1281, 1163 (C—O—C); ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data in $CDCl_3$, see Table 1; HR-ESI-MS *m/z* 382.1261 $[M + Na]^+$ (calcd for $C_{19}H_{21}NO_6Na$, 382.1267); CD (MeOH) $\Delta\varepsilon_{236}$, +5.33, $\Delta\varepsilon_{279}$, −21.13.

3.4 X-ray crystallographic data of 2

The single crystal of **2** with dimensions of 0.43 × 0.37 × 0.37 mm for X-ray diffraction was selected. Compound **2** crystallizes in the orthorhombic system, space group *P*2(1)2(1)2(1) with $a = 7.0328$ (13), $b = 13.296$ (3), $c = 18.662$ (4) Å, $V = 1745.1$ (6) Å^3, $Z = 4$, $Dx = 1.436$ g/cm^3, $F(000) = 800$, $\mu(MoK_\alpha) = 0.110$ mm^{-1}, the final $R = 0.0320$, and $wR = 0.0822$ for 2291 independent reflections with $R_{int} = 0.0299$ and 2218 observed reflections with $I > 2\sigma(I)$.

3.5 DPPH inhibition test

The inhibition effect of **1** against DPPH radical was evaluated according to spectrophotometric assay [8]. The absorbance of the test solution was determined at 517 nm on 96-well microplates and the percent of inhibition was calculated.

3.6 Neuroprotective effects

The neuroprotective effects of **2** against hydrogen peroxide and β-amyloid$_{25-35}$-induced oxidative injury were evaluated according to the reported protocol [9] with minor modification on PC-12 cells. Cell survival was evaluated by MTT reduction. The values of cell survival were normalized against the values for control group, which is set to 100%. Data are expressed as compound treated group subtract

H_2O_2/Aβ_{25-35}-injured group. Data were evaluated for statistical significance with one-way ANOVA followed by LSD test by using a computerized statistical package. Differences were considered significant at $P < 0.05$.

Acknowledgements

The authors are thankful for financial support from programs of Shaanxi Science and Technology Commission (No. 2010JM2014), Shaanxi Education Commission (No. 09JS067, 2010JK407), and Baoji Technology Department (No. 08SF01-2). The authors also thank Prof. Hai-Yang Zhang of Shanghai Institute of Materia Medica for the neuroprotective effects test.

References

[1] Jiangsu New Medical Colledge, *Dictionary of Chinese Medicine* (Shanghai Science and Technology Press, Shanghai, 1985), p. 1234.

[2] China Pharmacopoeia Committee, *China Pharmacopoeia, Part 1*, 2005 ed. (Chemical Industry Press, 2005), p. 135.

[3] L. Shu, W. Yin, J. Zhang, B. Tang, Y.X. Kang, F. Ding, and Z.C. Hua, *Cell Biol. Int.* **3**, 784 (2007).

[4] H.Z. Jin, X.L. Wang, H.B. Wang, Y.B. Wang, L.P. Lin, J. Ding, and G.W. Qin, *J. Nat. Prod.* **71**, 127 (2008).

[5] X.L. Wang, H.Z. Jin, Z.X. Li, and G.W. Qin, *Fitoterapia* **78**, 593 (2007).

[6] G.H. Bao, X.L. Wang, X.C. Tang, P. Chiu, and G.W. Qin, *Tetrahedron Lett.* **50**, 4375 (2009).

[7] G.H. Bao, G.W. Qin, R. Wang, and X.C. Tang, *J. Nat. Prod.* **68**, 1128 (2005).

[8] A. Moure, D. Franco, J. Sineiro, H. Domínguez, M.J. Núnez, and J.M. Lema, *J. Agric. Food Chem.* **48**, 3890 (2000).

[9] X.Q. Xiao, R. Wang, and X.C. Tang, *J. Neurosci. Res.* **61**, 564 (2000).