Morphinane Alkaloid Dimers from Sinomenium acutum

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Morphinane Alkaloid Dimers from *Sinomenium acutum*

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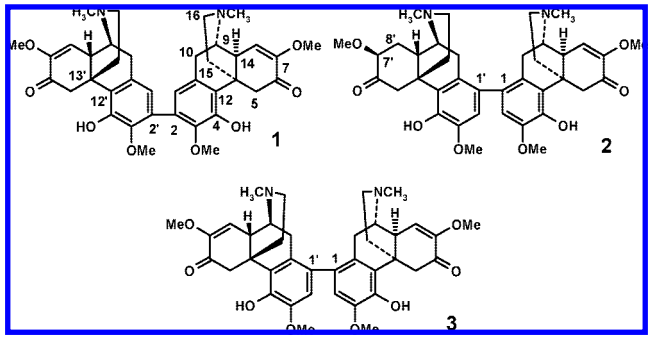
Two new morphinane alkaloid dimers, 2,2′-disinomenine (**1**) and 7′,8′-dihydro-1,1′-disinomenine (**2**), and known 1,1′-disinomenine (**3**), were isolated from ethanol extracts of stems of *Sinomenium acutum*. Their structures were elucidated on the basis of spectroscopic methods. The absolute configuration of alkaloids **1**−**3** was determined by direct comparison of their CD spectra with the known alkaloid sinomenine. The isolated alkaloids were tested for cytotoxicity against A549, P388, and HeLa cell lines, and **1** and **3** showed weak inhibition against A549 and Hela cells.

Sinomenium acutum (Thumb.) Rehd. et Wils. (Menispermaceae) is a deciduous liana, distributed widely in Central and South China. The dried stem of the plant is a traditional Chinese medicine, recorded in the Chinese Pharmacopoeia (2005 edition) for treatment of arthralgia due to pathogenic wind-dampness, arthral paralysis, and swelling.[1] The plant contains many bisbenzylisoquinoline, aporphine, oxoisoaporphine, protoberberine, and morphinane-type alkaloids. Sinomenine, the major alkaloid, exhibited a range of bioactivities including anti-inflammation, immunosuppression, arthritis amelioration, and protection against hepatitis induced by LPS.[2] Tablets of sinomenine hydrochloride, an approved drug, have been used in China for treatment of acute rheumatoid arthritis.[3] Our studies of sinomenine and its derivatives have shown them to possess cognitive-enhancing activities in three animal models: water maze in mice, social recognition in rats, and object recognition in rats.[4] We also reported that *N*-demethyl sinomenine and sinoacutine have protective effects against hydrogen peroxide-induced cell injury.[5] In our continuing study, two new morphinane alkaloid dimers, 2,2′-disinomenine (**1**) and 7′,8′-dihydro-1,1′-disinomenine (**2**), together with known 1,1′-disinomenine (**3**) were isolated from an ethanol extract of the stems of *S. acutum*. We herein report the isolation, structural elucidation, and the results of cytotoxicity tests of these compounds against several tumor cell lines.



Compound **1** was obtained as white needles. Its molecular formula was determined to be $C_{38}H_{44}N_2O_8$ on the basis of HREIMS (*m*/*z* 656.3079 $[M]^+$, calcd 656.3098). The IR spectrum showed characteristic absorptions for OH (3432 cm^{-1}), α,β-unsaturated carbonyl (1683, 1629 cm^{-1}), and substituted phenyl (1598, 1465 cm^{-1}) groups. Analysis of ^1H and ^{13}C NMR spectra together with DEPT and HSQC experiments revealed signals for 19 carbons and 22 protons (Tables 1 and 2), only half of the numbers expected from the molecular formula, indicating that **1** should be a symmetric alkaloid dimer. The ^{13}C NMR spectrum showed 19 signals for three methyl (all oxygenated and/or nitrogenated), four methylene, four methine (one aromatic, one olefinic), and eight quaternary (one carbonyl, six aromatic) carbons. The ^1H NMR spectrum displayed signals for two methoxyl (δ_H 3.76, 3.51, each 3H, s), one N-methyl (δ_H 2.31, 3H, s), nine methylene, and eight methine protons. The ^1H and ^{13}C NMR data of **1** were very similar to those of sinomenine,[5] except for the change from two ortho-coupled aromatic protons (δ_H 6.56, 6.68, each 1H, d, J = 8.2 Hz) in sinomenine to an isolated proton (δ_H 6.44, 1H, s) in **1**, indicating that two monomers should be coupled by C-1−C-1′ or C-2−C-2′. The HMBC experiment showed the cross-peaks of H-10 (or H-10′) to C-1 (or C-1′); H-1 (or H-1′) to C-2 (or C-2′); and H-1 (or H-1′) to C-10 (or C-10′), suggesting the C-2/C-2′ linkage. The ROESY spectrum displayed cross-peaks of H-5β (or H-5′β)/H-14 (or H-14′); H-5α (or H-5′α)/H-15α (or H-15′α), and H-14 (or H-14′)/H-15β (or H-15′β), indicating that H-14 (or H-14′) and the N-containing ring were on the same side, having an α-orientation.[6] Furthermore, the circular dichroism (CD) spectrum of **1** showed negative ($\Delta\epsilon$, −14.13) and positive ($\Delta\epsilon$, +19.33) Cotton effects at 273 and 234 nm, respectively, similar to those of sinomenine (Figure 1), suggesting that the absolute configuration of **1** was the same as that of sinomenine. Thus, **1** was determined to be 2,2′-disinomenine.

Compound **2** was obtained as an amorphous powder. Its molecular formula was determined by HREIMS (*m*/*z* 658.3259 $[M]^+$, calcd 658.3254) as $C_{38}H_{46}N_2O_8$, implying that **2** was a dihydro derivative of **1**. The ^{13}C NMR spectrum together with DEPT and HMQC experiments exhibited 38 signals (Table 2) for six methyl (all oxygenated and/or nitrogenated), nine methylene, eight methine (two aromatic, one olefinic, one oxygenated), and 15 quaternary (two carbonyl, 11 aromatic) carbons. The ^1H NMR spectrum (Table 1) displayed signals for four methoxyl (δ_H 3.80, 3.79, 3.54, 3.53, each 3H, s), two N-methyl (δ_H 2.34, 2.31, each 3H, s), nine methylene, and eight methine (two aromatic at δ 6.38, 6.47, each 1H, s; one olefinic at δ 5.37, d, J = 2.0 Hz) protons. The ^1H and ^{13}C NMR spectra of **2** were similar to those of **1**. However, the ^1H and ^{13}C NMR signals of **2** were divided into two groups, indicating that**2** was an asymmetric sinomenine-related dimer. Further study of NMR data suggested that one monomer in **2** was sinomenine and that the other was 7′,8′-dihydro-sinomenine. One set of olefinic signals (C-7′, C-8′, and H-8′) was absent, and an oxygrnated methane (C-7′ and H-7′) and an additional methylene appeared. Key HMBC correlations between H-2/C-1′, C-4, C-11; H-10/C-1, C-12, C-14; H-2′/C-1, C-4′, C-11′; and H-10′/C-1′, C-14′ were observed, suggesting the C-1/C-1′ linkage of the two monomers. From the J value of H-7′ with H_2-8′ (dd, J = 10.0, 6.8

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Table 1. ^{1}H NMR (400 MHz, $CDCl_3$) Spectroscopic Data for **1**−**3**

position	**1**	**2**	**3**
1	6.44 (s)		
2		6.38 s	6.26 s
5α	2.48 (d 15.6)	2.47 (d 15.6)	2.47 (d 15.2)
5*β*	4.42 (d 15.6)	4.39 (d 15.6)	4.40 (d 15.2)
8	5.27 (d 2.0)	5.37 (d 2.0)	5.43 (d 2.0)
9	2.98 (t 2.0)	3.09 (t 4.0)	3.08 (t 4.0)
10α	2.53 (d 18.8)	2.62 (d 18.8)	2.32 (d 18.0)
10*β*	1.75 (dd 5.2 18.8)	1.80 (dd 18.8, 5.2)	2.39 (dd 5.2,18.0)
14	2.99 (br d 2.0)	2.98 br s	2.99 br s
15α	2.01 (dd 3.7, 12.4)	2.01 m	2.01 (dd 2.4,12.4)
15*β*	1.91 (dd 4.0, 12.4)	1.92 m	1.92 (ddd 4.4,6.0,12.4)
16α	2.05 dd 3.7, 11.8)	2.09 m	2.17 (ddd 2.4,6.0,12.4)
16*β*	2.57 dd 4.0,11.8)	2.58 m	2.55 (dd 4.4,12.4)
NMe	2.31 (s)	2.34 s	2.33 s
3-OMe	3.76 (s)	3.78 s	3.73 s
7-OMe	3.51 (s)	3.44 s	3.50 s
1′	6.44 (s)		
2′		6.47 s	6.26 s
5′α	2.48 (d 15.6)	2.29 (d 15.2)	2.47 (d 15.2)
5′*β*	4.42 (d 15.6)	4.37 (d 15.2)	4.40 (d 15.2)
7′		3.92 (dd 10.0, 6.8)	
8′α	5.27 (d 2.0)	1.53 (dd 12.4,10.0)	5.43 (d 2.0)
8′*β*		2.07 m	
9′	2.98 (t 2.0)	2.81 br s	3.08 (t 4.0)
10′α	2.53 (d 18.8)	2.52 m	2.32 (d 18.0)
10′*β*	1.75 (dd 5.2 18.8)	2.48 m	2.39 (dd 5.2,18.0)
14′	2.99 (br d 2.0)	2.35 m	2.99 br s
15′α	1.91 (dd 4.0, 12.4)	2.00 m	2.01 (dd 2.4,12.4)
15′*β*	2.01 (dd 3.7, 12.4)	1.99 m	1.92 (ddd 4.4,6.0,12.4)
16′α	2.05 dd 3.7, 11.8)	2.06 m	2.17 (ddd 2.4,6.0,12.4)
16′*β*	2.57 dd 4.0,11.8)	2.57 m	2.55 (dd 4.4,12.4)
NMe	2.31 (s)	2.31 s	2.33 s
3′-OMe	3.76 (s)	3.81 s	3.73 s
7′-OMe	3.51 (s)	3.46 s	3.50 s

Hz), H-7′ should be axial. The CD spectrum of **2** showed negative ($\Delta\epsilon$, −12.02) and positive ($\Delta\epsilon$, +22.55) Cotton effects at 273 and 233 nm, respectively, similar to those of sinomenine (Figure 1), suggesting that the absolute configuration of **2** was the same as that of sinomenine. Therefore, the structure of **2** was established as 7′,8′-dihydro-1,1′-disinomenine.

The known alkaloid dimer 1,1′-disinomenine (**3**) was obtained as white needles. Its HREIMS indicated the molecular formula $C_{38}H_{44}N_2O_8$, the same as that of **1**. Natural 1,1′-disinomenine dimer has been suggested to be an oxidative product of sinomenine.[7] This current study is the first to completely assign all of the ^{1}H and ^{13}C NMR signals of **3** (Tables 1 and 2). HMBC correlations between H-2/C-11, C-1′, C-4 and H-10/C-1, C-12 indicated the C-1/C-1′ linkage of two sinomenine units. The CD spectra of **3** showed Cotton effects at 273 and 233 nm, respectively, similar to those of sinomenine (Figure 1), suggesting that the absolute configuration of **3** was the same as that of sinomenine.

It was reported earlier that morphine can be dimerized to 2,2′-dimorphine by oxidation in air.[8] The new morphinane alkaloid dimers 2,2′-disinomenine (**1**) and 7′,8′-dihydro-1,1′-disinomenine (**2**) together with known 1,1′-disinomenine (**3**) could also have been produced by slow autoxidation.

Alkaloids **1**−**3** were evaluated for cytotoxicity against A549, P388, and HeLa cell lines using the MTT assay.[9] The results showed that **1** and **3** had weak cytotoxic activities against A549 cells with IC_{50} values of 65.50 and 71.83 μM and against Hela cells with IC_{50} values of 71.70 and 70.69 μM, respectively.

Experimental Section

General Experimental Procedures. Melting points were determined on a XT-4 microscopic thermometer without correction. Optical rotations were determined on a Perkin-Elmer 341 polarimeter. UV spectra were obtained on a UV-1601 UV–vis spectrophotometer. IR spectra were recorded on a Magna-IR 750 spectrometer in KBr pellets. CD spectra were measured on a JASCO J-810 spectrometer in MeOH, and data are given as $\Delta\epsilon$ (nm). NMR spectra were measured on Varian Mercury-400 and Innova-600 instruments. EIMS was obtained on a Finnigan MAT95 mass spectrometer. All solvents used were of analytical grade (Shanghai Chemical Company, Ltd.). Silica gel (200–300 mesh) was used for column chromatography, and precoated silica GF_{254} plates used for TLC (Qingdao Haiyang Chemical Company, Ltd.).

Plant Material. The stems of *S. acutum* were collected in the Qin Ling Mountains, Shaanxi Province, China, in August 2003 and identified by Prof. Zhijun Fu, Department of Biology, Baoji College of Arts and Sciences. A voucher specimen (No. SIMM0004) was deposited in the Shanghai Institute of Materia Medica.

Extraction and Isolation. The stems of *S. acutum* (20 kg) were extracted twice with ethanol at room temperature. The ethanol extract (800 g) was suspended in aqueous MeOH and partitioned with cyclohexane. The aqueous layer was then evaporated to afford an extract (600 g). A part of the extract (100 g) was chromatographed on a silica gel column (7 × 40 cm) eluting with $CHCl_3$−MeOH (100:0, 50:1, 10:1, 5:1, 1:1, MeOH) to afford six fractions (1–6). Fraction 3 was subjected to silica gel column chromatography eluting with $CHCl_3$−MeOH (10:1−1:1 gradient) to afford eight subfractions (31–38). The latter were combined and subjected to silica gel chromatography with $CHCl_3$−MeOH (10:1) to obtain compound **1** (20.2 mg). Fraction 4 was subjected to silica gel column chromatography eluting with $CHCl_3$−MeOH (5:1) to afford nine subfractions (41–49). Fraction 48 was chromatographed on silica gel using acetone−MeOH (1:1) to afford four fractions (481–484). Fraction 482 was crystallized to recover compound **3** (40.1 mg). Fraction 484 was subjected to silica gel eluting with $CHCl_3$−MeOH (5:1) to obtain compound **2** (3.0 mg).

2,2′-Disinomenine (1): white needles; mp 214–215 °C; $[\alpha]^{25}_D$ +48 (*c* 0.16, $CHCl_3$); UV (MeOH) λ_{max} 208, 294 nm; IR (KBr) ν_{max} 3432, 2931, 1683, 1629, 1598, 1465, 1415, 1319, 1274, 1200, 1147, 860 cm^{-1}; CD (MeOH) 234 ($\Delta\epsilon$, +19.33), 273 ($\Delta\epsilon$, −14.13) nm; ^{1}H NMR and ^{13}C NMR, see Tables 1 and 2; HREIMS *m*/*z* 656.3079 $[M]^+$ (calcd for $C_{38}H_{44}N_2O_8$, 656.3098).

7′,8′-Dihydro-1,1′-disinomenine (2): amorphous powder; mp 220–221 °C; $[\alpha]^{25}_D$ +79 (*c* 0.06, MeOH); UV (MeOH) λ_{max} 211, 290 nm; IR (KBr) ν_{max} 3430, 2935, 1681, 1629, 1598, 1465, 1438, 1276,

Table 2. ^{13}C NMR (100 MHz, $CDCl_3$) Spectroscopic Data for **1**−**3**

position	1	2	3
1	109.32	130.85	130.53
2	130.84	109.34	110.64
3	145.06	144.99	144.84
4	143.90	143.92	143.73
5	49.07	49.11	49.21
6	193.86	194.01	193.94
7	152.34	152.29	152.38
8	114.49	114.88	115.64
9	56.34	55.97	56.22
10	22.48	22.73	23.76
11	127.93	128.24	127.66
12	122.95	123.21	123.22
13	40.55	40.59	40.79
14	45.46	45.54	45.62
15	35.63	35.66	35.78
16	47.28	47.34	47.11
NMe	43.09	42.96	42.90
3-OMe	56.06	56.07	55.88
7-OMe	54.37	54.59	54.77
1′	109.32	131.24	130.53
2′	130.84	109.79	110.64
3′	145.06	145.25	144.84
4′	143.90	144.07	143.73
5′	49.07	48.95	49.21
6′	193.86	207.58	193.94
7′	152.34	83.58	152.38
8′	114.49	34.78	115.64
9′	56.34	56.67	56.22
10′	22.48	22.09	23.76
11′	127.93	127.58	127.66
12′	122.95	122.48	123.22
13′	40.55	42.02	40.79
14′	45.46	44.01	45.62
15′	35.63	38.00	35.78
16′	47.28	46.72	47.11
NMe	43.09	42.96	42.90
3′-OMe	56.06	56.17	55.88
7′-OMe	54.37	58.31	54.77

1201, 1108, 858 cm^{-1}; CD (MeOH) 233 ($\Delta\epsilon$, +22.55), 273 ($\Delta\epsilon$, −12.02) nm; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; HREIMS *m*/*z* 658.3259 $[M]^+$ (calcd for $C_{38}H_{46}N_2O_8$, 658.3254).

1,1′-Disinomenine (3): white needles; mp 209–210 °C; $[\alpha]^{25}_D$ +78 (*c* 0.36, $CHCl_3$); UV (MeOH) λ_{max} 212, 256, 293 nm; IR (KBr) ν_{max} 3425, 2935, 1687, 1627, 1598, 1463, 1438, 1274, 1201, 1147, 1054, 889 cm^{-1}; CD (MeOH) 233 ($\Delta\epsilon$, +13.61), 274 ($\Delta\epsilon$, −14.50) nm; ^1H NMR and ^{13}C NMR, see Tables 1 and 2; HREIMS *m*/*z* 656.3099 $[M]^+$ (calcd for $C_{38}H_{44}N_2O_8$, 656.3098).

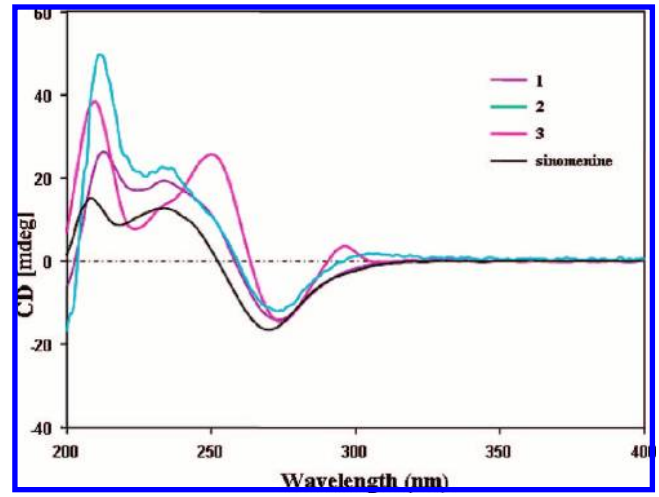

Figure 1. CD spectra of **1**−**3** and sinomenine.

Cytotoxicity Assay. The cytotoxicity assay was carried out according to a procedure described in the literature.[9]

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