JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Cyclopeptide Alkaloids from *Ziziphus apetala*

Jing Han,[†,∥,‡] Chang-Jiu Ji,[†,∥,‡] Wen-Jun He,[†] Yu Shen,[§,∥] Ying Leng,[§] Wen-Yan Xu,[†] Jun-Ting Fan,[†] Guang-Zhi Zeng,[†] Ling-Dong Kong,[⊥] and Ning-Hua Tan*[,†]

[†]State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, People's Republic of China

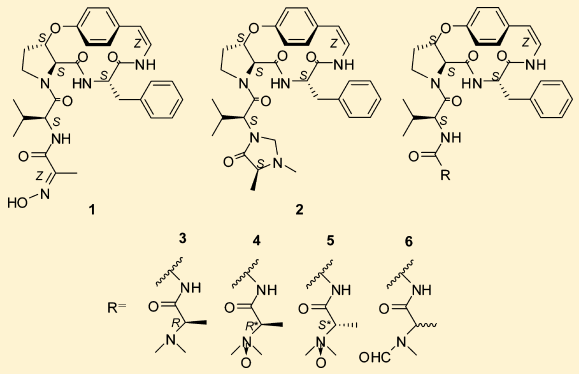
[§]Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, People's Republic of China

[⊥]Nanjing University, Nanjing 210093, People's Republic of China

[∥]Graduate University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

Supporting Information

ABSTRACT: Six novel Ia_3-type cyclopeptide alkaloids (**1**−**6**) were isolated from stems of *Ziziphus apetala*. Compound **5** and the known compounds mauritine A (**7**) and mauritine F (**8**) were isolated from the roots. Their structures were determined by spectroscopic analyses and chemical methods. The total alkaloids from the roots and the isolated cyclopeptide alkaloids were tested for antidepressant behavior on mice, cytotoxicity, and 11β-hydroxysteroid dehydrogenase (11β-HSD) inhibition in vitro. Only mauritine A (**7**) showed inhibitory activity on 11β-HSD1, with IC_{50} values of 52.0 (human) and 31.2 μg/mL (mouse).



The plant genus *Ziziphus* (Rhamnaceae) is distributed throughout China and has commonly been used in traditional Chinese medicine for treatment of various diseases and for insomnia.[1] Cyclopeptide alkaloids (CPAs) are characteristic components of *Ziziphus* plants and are heteromonocyclopeptides with a 13-, 14-, or 15-membered ring. The compounds usually embody a *p*- or *m*-ansa structure with one styrylaminine moiety and contain two or three α-amino acid residues.[2] The 14-membered ring CPAs are divided into three types (Ia, Ib, and Ic). The Ia type is the largest group, which includes three subtypes (Ia_1−Ia_3). Over 100 CPAs have been isolated from *Ziziphus* species since the first *Ziziphus* CPA, zizyphine-A, was reported by Ménard in 1963.[3] Some CPAs have exhibited sedative, antibacterial, and antifungal activities.[2,4]

Fruits of *Ziziphus apetala* Hook. F. (Rhamnaceae) have been used as a folk herb to relieve symptoms of eczema and skin allergies. There is no literature relating to chemical constituents of this plant. Herein we report six new Ia_3-type CPAs (**1**−**6**) isolated from the stems of *Z. apetala*, as well as **5** and the known compounds mauritine A (**7**)[5] and mauritine F (**8**)[5] from the roots. The total alkaloids from the roots and the isolated CPAs were tested for antidepressant behavior on mice, cytotoxicity, and 11β-hydroxysteroid dehydrogenase (11β-HSD) inhibition in vitro.

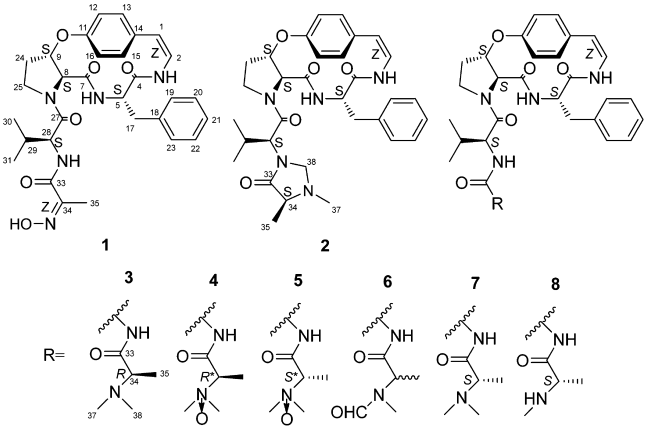
RESULTS AND DISCUSSION

Air-dried and powdered stems of *Z. apetala* were extracted with 95% EtOH. The alkaloid fraction was then prepared using the acid−base method. This alkaloid fraction was fractionated on a silica gel column eluted with increasingly polar mixtures of $CHCl_3$/MeOH. Further purification was achieved by chromatography on Sephadex LH-20 ($CHCl_3$/MeOH), RP-18 (MeOH/H_2O), and HPLC (CH_3CN/H_2O containing 0.4 ‰ TFA), to yield compounds **1**−**6**.

Compound **1** was obtained as a white powder, $[\alpha]^{22}_D$ −19.3 (*c* 0.30, MeOH), which had the molecular formula $C_{30}H_{35}N_5O_6$ by HRESIMS (*m*/*z* 584.2481 $[M + Na]^+$), indicating 16 degrees of unsaturation. The IR spectrum indicated the presence of amino (3424 cm^{-1}), amide (1676 cm^{-1}), styryl double bond (1628 cm^{-1}), and phenol ether (1210 and 1186 cm^{-1}) groups. The ^{13}C NMR spectrum revealed that there were no *N*-dimethyl or *N*-monomethyl terminal amino acid residues, but that it had one each quaternary olefinic (δ_C 151.0), carbonyl (δ_C 165.1), and methyl (δ_C 10.0) signals that were not present in the spectrum of mauritine A (**7**).[5] The ^1H NMR spectrum presented an OH signal at δ_H 14.20 and a methyl signal at δ_H 2.35. These data suggested a possible CO−$C(CH_3)$=N−OH segment (2-(hydroxyimino)propanoic acid). Correlations of H-3 to C-4, H-6 to C-7, and H-9 to C-11 in the HMBC spectrum indicated connections between *p*-oxystyrylamino and phenylalanine, phenylalanine and β-oxyproline, and β-oxyproline and *p*-oxystyrylamino moieties, i.e., the 14-menbered ring. The cross-peak of H-28 to C-33 confirmed the connection of valine and 2-(hydroxyimino)-propanoic acid. Interaction between H-25 and H-28 observed

Received: September 20, 2011
Published: December 7, 2011

in the ROESY spectrum supported the linkage of valine and β-oxyproline. Thus, the planar structure of **1** was determined to belong to the Ia_3-type CPAs. Interactions of H-8 with H-12 and of H-9 with H-16 in the ROESY implied that H-15 and H-16 are oriented upward, while H-12 and H-13 are oriented downward.[6] Amino acid analysis of the hydrolysate of **1** showed an *S* configuration of C-5 (L-Phe) and an *S* configuration of C-28 (L-Val) according to Marfey's method.[7] The *J* value of H-1 (δ_H 6.53, d, 7.4) and H-2 (δ_H 6.63, t, 7.4) indicated the *Z* configuration of the double bond, which was confirmed by their obvious interactions in the ROESY spectrum.[8] In the ROESY spectrum, no NOE interaction between H-5 and H-8 supported an *S* configuration of C-8 (Pro). No NOE interaction between H-8 and H-9, together with the *J* value of H-8 (6.3 Hz), indicated the *S* configuration[8] of C-9 (β-C of β-oxyproline), similar to mauritine A (**7**).[6] The OH signal at very low field indicated strong intramolecular hydrogen bonding of the OH and C═O groups of the 2-(hydroxyimino)propanoic acid moiety, and no NOE interaction between the OH and H-35 suggested the *Z* configuration of the carbon−nitrogen double bond. Compound **1** was named apetaline A.



The molecular formula of compound **2** was determined to be $C_{32}H_{39}N_5O_5$ by HRESIMS at *m/z* 596.2858 $[M + Na]^+$, indicating 16 degrees of unsaturation. Comparison of the ^{13}C NMR spectrum of **2** (Table 1) with that of **1** showed differences only in the terminal amino acid residue. Correlation of H-34 and H-35 in the COSY, the connections of H-34 and H-38 to C-33 in the HMBC, and 2 degrees of unsaturation suggested that **2** had a five-membered ring formed by one methyl (C-38) of *N*,*N*-dimethylalanine and the NH-32 of valine as the N-terminal residue imidizolidine-4-one.[9] HMBC correlations were observed for H-3/C-4, H-6/C-7, and H-9/C-11, indicating the 14-membered ring. Cross-peaks of H-8 and H-28 with C-27 in the HMBC and NOE interactions between H-25 and H-28 revealed that the valine residue was attached to the β-oxyproline unit. The interaction of H-28 with C-33 in the HMBC indicated the connection between valine and the N-terminal imidizolidine-4-one. Thus, as for **1**, the absolute configurations of C-5, C-8, C-9, and C-28 were identified as *S*, *S*, *S*, and *S*, with a *Z* double bond between C-1 and C-2. Interactions of H-34 with H-38b and H-37, H-38b with H-28, and H-28 with H-25 in the ROESY spectrum suggested the *S* configuration of C-34. Compound **2** was named apetaline B.

The molecular formula of compound **3** was determined to be $C_{32}H_{42}N_5O_5$, indicating 15 degrees of unsaturation. The ^1H NMR and ^{13}C NMR spectra (Tables 1 and 2, Supporting Information) were similar to those of mauritine A (**7**),[5] only the ^{13}C NMR signals of the terminal *N*,*N*-dimethylalanine, i.e., the carbonyl (C-33, δ_C 167.4) (174.3 for **7**), α-animo methine (C-34, δ_C 62.3) (65.5), and *N*-methyl (C-37/38, δ_C 40.1) (42.5) signals, differed. Compounds **3** and **7** had different retention times of the terminal *N*,*N*-dimethylalanine according to the animo acid analysis, i.e., **3** (D, rt 27.66 min), **7** (L, rt 38.17 min). Correlations of H-3/C-4, H-6/C-7, and H-9/C-11 in the HMBC confirmed the 14-membered ring. The HMBC cross-peaks of H-8 and H-28 to C-27 and H-28 to C-33, with a NOE interaction of H-8 to H-28, indicated linkage of β-oxyproline with valine, and valine with *N*,*N*-dimethylalanine. The absolute configurations of C-5, C-8, C-9, and C-28 were identified as *S*, *S*, *S*, and *S* with a *Z* double bond between C-1 and C-2. Compound **3** was the C-34 epimer of **7**.

Compounds **4** and **5** had the same molecular formula ($C_{32}H_{41}N_5O_6$) by positive HRFABMS, with 15 degrees of unsaturation, one more oxygen than mauritine A (**7**).[5] ^{13}C NMR data (Table 1, Supporting Information) of the terminal amino acid residue differed from **7** as follows: upfield signals of carbonyl (C-33, δ_C 169.9/170.7) (174.3 for **7**); downfield signals of α-amino methine (C-34, δ_C 74.5/74.1) (65.5), and *N*-methyl {(C-37 and -38, δ_C 56.8 and 56.7)/(58.0 and 57.4)} (42.5), which indicated an unusual *N*-oxide group.[10] Compounds **4** and **5** are epimers at C-34. In accordance with the ^{13}C NMR signals of the terminal amino acid, compared with compounds **3** and **7**, having *R* and *S* configuration at C-34, **4** was assigned an *R** configuration at C-34 and **5** was assigned an *S** configuration at C-34. HMBC correlations of H-3/C-4, H-6/C-7, and H-9/C11 in **4** and **5** confirmed the 14-membered ring. Cross-peaks of H-8 and H-28 to C-27 and H-28 to C-33 in the HMBC, together with a NOE interaction of H-8 with H-28, indicated the linkage of β-oxyproline with valine, and valine with oxide-*N*,*N*-dimethylalanine. The absolute configurations at C-5, C-8, C-9, and C-28 were identified as *S*, *S*, *S*, and *S* with a *Z* double bond between C-1 and C-2 by methods similar to those used for **1**. Compounds **4** and **5** were the *N*-oxides of **3** and **7**.

The molecular formula of compound **6** was determined to be $C_{32}H_{39}N_5O_6$ by positive HRESIMS *m/z* 612.2782 $[M + Na]^+$, indicating 16 degrees of unsaturation. The NMR spectral data (Tables 1 and 2, Supporting Information) were similar to those of mauritine F (**8**),[5] except the ^{13}C NMR signals of the terminal amino acid were upfield, together with the cross-peaks of H-34 and H-38 to C-37 in the HMBC, indicating the existence of a $CO-CH(CH_3)-N(CH_3)-CHO$ segment as *N*-formylmonomethylalanine.[11] HMBC correlations of H-3/C-4, H-6/C-7, and H-9/C11 in **6** confirmed the 14-membered ring. Cross-peaks of H-8 and H-28 to C-27 and H-28 to C-33 in the HMBC, together with a NOE interaction of H-8 to H-28, indicated linkage of β-oxyproline with valine, and valine with *N*-formylmonomethylalanine. According to the methods used for the previous compounds, the absolute configurations of C-5, C-8, C-9, and C-28 were identified as *S*, *S*, *S*, and *S* with a *Z* double bond between C-1 and C-2. However, the absolute configuration of C-34 could not be determined. Compound **6** was named apetaline C.

Apetaline A (**1**) has an uncommon oximinoketone segment in the terminal side chain. Apetaline B (**2**) contained an N-terminal imidizolidine ring. Compounds **4** and **5** are N-oxygenated derivatives of **3** and **7**. Apetaline C (**6**) contained an *N*-formyl group. Compounds **3** and **4** are isomers of compounds **7** and **5**. The CD spectra displayed one main negative Cotton effect band around 230 nm, consistent with

dx.doi.org/10.1021/np200755t | J. Nat. Prod. 2011, 74, 2571−2575

Table 1. ^{13}C NMR Data (δ) for Compounds 1−6

no.	1[b,d]	1[c,e]	2[c,d]	3[b,e]	4[b,d]	5[c,d]	6[a,e]
1	122.8, CH	116.0, CH	122.4, CH	115.8, CH	122.3, CH	122.1, CH	114.8, CH
2	127.0, CH	125.3, CH	126.9, CH	125.3, CH	127.0, CH	126.9, CH	125.3, CH
4	169.4, qC	166.6, qC	169.1, qC	166.9, qC	169.2, qC	169.1, qC	165.5, qC
5	55.7, CH	54.3, CH	55.5, CH	54.1, CH	55.6, CH	55.5, CH	54.0, CH
7	172.0, qC	173.1, qC	171.6, qC	170.3, qC	171.8, qC	171.9, qC	170.2, qC
8	66.2, CH	64.7, CH	65.9, CH	64.2, CH	66.0, CH	66.0, CH	64.1, CH
9	84.3, CH	84.1, CH	84.2, CH	83.8, CH	84.2, CH	84.2, CH	83.7, CH
11	158.4, qC	157.4, qC	158.2, qC	157.3, qC	158.3, qC	158.2, qC	157.3, qC
12	122.5, CH	122.2, CH	122.2, CH	122.5, CH	122.3, CH	122.3, CH	122.6, CH
13	130.8, CH	130.3, CH	130.6, CH	130.2, CH	130.7, CH	130.7, CH	130.3, CH
14	132.9, qC	132.6, qC	132.8, qC	132.5, qC	132.8, qC	132.8, qC	132.5, qC
15	132.2, CH	132.3, CH	132.2, CH	132.2, CH	132.2, CH	132.2, CH	132.4, CH
16	120.7, CH	121.9, CH	120.5, CH	122.1, CH	120.7, CH	120.7, CH	122.4, CH
17	38.5, CH_2	36.6, CH_2	38.5, CH_2	36.3, CH_2	38.3, CH_2	38.2, CH_2	36.1, CH_2
18	138.5, qC	135.6, qC	138.7, qC	135.7, qC	138.4, qC	138.3, qC	135.3, qC
19	130.3, CH	129.7, CH	130.1, CH	129.7, CH	130.3, CH	130.3, CH	130.0, CH
20	128.7, CH	128.6, CH	128.6, CH	128.5, CH	128.7, CH	128.6, CH	128.7, CH
21	126.9, CH	127.0, CH	126.7, CH	127.0, CH	126.8, CH	126.7, CH	127.2, CH
22	128.7, CH	128.6, CH	128.6, CH	128.5, CH	128.7, CH	128.6, CH	128.7, CH
23	130.3, CH	129.7, CH	130.1, CH	129.7, CH	130.3, CH	130.3, CH	130.0, CH
24	32.3, CH_2	31.8, CH_2	32.0, CH_2	31.8, CH_2	32.3, CH_2	32.3, CH_2	31.9, CH_2
25	46.5, CH_2	46.9, CH_2	46.4, CH_2	46.5, CH_2	46.4, CH_2	46.4, CH_2	46.5, CH_2
27	171.5, qC	170.1, qC	169.1, qC	170.6, qC	171.3, qC	171.5, qC	172.5, qC
28	55.9, CH	56.3, CH	57.3, CH	56.1, CH	56.7, CH	56.4, CH	54.9, CH
29	32.0, CH	30.9, CH	28.7, CH	30.6, CH	31.0, CH	30.9, CH	31.5, CH
30	19.7, CH_3	19.3, CH_3	19.0, CH_3	18.9, CH_3	19.5, CH_3	19.5, CH_3	19.2, CH_3
31	18.4, CH_3	18.9, CH_3	18.7, CH_3	18.2, CH_3	18.9, CH_3	18.7, CH_3	17.8, CH_3
33	165.1, qC	164.5, qC	174.2, qC	167.4, qC	169.9, qC	170.7, qC	170.2, qC
34	151.0, qC	151.6, qC	62.5, CH	62.3, CH	74.5, CH	74.1, CH	50.2, CH
35	10.0, CH_3	9.9, CH_3	15.6, CH_3	13.1, CH_3	13.8, CH_3	13.9, CH_3	12.8, CH_3
37			38.7, CH_3	40.1, CH_3	56.8, CH_3	58.0, CH_3	163.3, CH
38			67.7, CH_2	40.1, CH_3	56.7, CH_3	57.4, CH_3	30.8, CH_3

[a]Recorded at 150 MHz. [b]Recorded at 125 MHz. [c]Recorded at 100 MHz. [d]Recorded in pyridine-d_5. [e]Recorded in chloroform-d_1.

the 5*S*, 8*S*, 9*S* configuration.[12] The positive ion mass spectra gave molecular ion peaks and characteristic fragmentation ions at *m/z* 446 (**1**), *m/z* 378 (**1**, **2**, **4**, **5**, **7**, **8**), *m/z* 292 (**2**, **3**), *m/z* 264 (**2**), *m/z* 221 (**3**), *m/z* 203(**4**, **5**), *m/z* 199 (**7**), and *m/z* 195 (**2**).

Mauritine A (**7**) was tested for in vitro inhibition of human and murine 11β-HSD1 and 11β-HSD2 enzymes, which showed inhibitory activities only on 11β-HSD1, with IC_{50} values of 52.0 (for human) and 31.2 (for mouse) μg/mL. The mixture of alkaloids from the roots was evaluated for antidepressant activity on mice, but showed no activity in forced swimming, tail suspension, or open-field tests. Compounds **1**−**8** were tested for cytotoxic activity against HeLa and BGC-823 cancer cell lines, but none exhibited cytotoxicity.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were obtained on an X-4 micromelting point apparatus. Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. IR spectra were obtained on a Tenor 27 spectrophotometer with KBr pellets. CD spectra were measured using a Chirascan spectrophotometer. 1D and 2D NMR spectra were run on Bruker AV-600, DRX-500, and AM-400 spectrometers with TMS as internal standard. Mass spectra were recorded on a VG Autospec-3000 spectrometer or an API QSTAR Pulsar TOF spectrometer. Analytical or semipreparative HPLC was performed on an Agilent 1100 with Zorbax Eclipse-C_{18} (4.6 mm × 150 mm; 9.4 mm × 250 mm; 5 μm). Column chromatography (CC) was performed on silica gel (200−300 mesh, Qingdao Yu-Ming-Yuan Chemical Co. Ltd., Qingdao, China), Sephadex LH-20 (Pharmacia Fine Chemical Co., Uppsala, Sweden), or Lichroprep RP-18 gel (40−63 μM, Merck, Darmstadt, Germany). Fractions were monitored by TLC (GF254, Qingdao Yu-Ming-Yuan Chemical Co. Ltd., Qingdao, China), and spots were detected by spraying with Dragendorff's reagent for alkaloids and ninhydrin reagent for cyclopeptides.[13]

Plant Material. The stems and roots of Z. *apetala* were collected from Mengla, Yunnan Province, PRC, in October 2008. The material was identified by Prof. Zhe-Kun Zhou at Kunming Institute of Botany. The voucher specimen (No. 4146) has been deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. Air-dried and powdered stems (28.0 kg) were extracted four times with 95% EtOH (4 L). The combined extract was concentrated under reduced pressure to 3.0 kg. The concentrated extract was dissolved in H_2O (500 mL) and acidified three times with 2 N HCl (3.6%, 2.5 L) to pH 2−3. The acidic solution was exhaustively extracted with EtOAc (5 × 500 mL) to yield 50 g of EtOH extract. The aqueous solution was basified with NaOH to pH 8−10 and extracted with $CHCl_3$ (5 L) to provide the alkaloid fraction (300 g). This alkaloid fraction was loaded onto a silica gel column and eluted with increasingly polar $CHCl_3$/MeOH (30:1−1:1). Seven major fractions (I−VII) were obtained, and fractions III and VI contained cyclopeptide alkaloids. Fraction III (100 g) was subjected to Sephadex LH-20 ($CHCl_3$/MeOH, 1:1 and 2:1), then Lichroprep RP-18 gel (MeOH/H_2O, 70:30−100:0), and further purified by HPLC (Zorbax Eclipse-C_{18}, 5 μM, 9.4 mm × 250 mm, 1.0 mL/min, UV

dx.doi.org/10.1021/np200755t | J. Nat. Prod. 2011, 74, 2571−2575

Table 2. ^{1}H NMR Data for Compounds 1−6

	1[a,d]	1[c,e]	2[b,d]	3[b,e]	4[b,d]	5[b,d]	6[a,e]
no.	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)
1	6.53, d, (7.4)	6.21, d, (8.2)	6.54, d, (7.5)	6.32, d, (8.0)	6.53, d, (7.0)	6.49, d, (7.4)	6.30, d, (7.9)
2	6.63, t, (7.4)	6.65, dd, (9.8, 8.2)	6.65, d, (7.5)	6.66, dd, (9.8, 8.0)	6.64, t, (7.0)	6.68, t, (7.4)	6.71, dd, (10.5, 7.9)
3	8.16, d, (7.4)	6.26, d, (9.8)		6.36, d, (9.8)	8.11, d, (7.0)	7.98, d, (7.4)	6.32, d, (10.5)
5	5.11, dt, (8.4, 4.5)	4.57, m	5.10, dd, (7.6, 4.6)	4.57, m	5.09, m	5.09, m	4.60, m
6	8.97, d, (8.4)	6.34, d, (7.7)		6.53, d, (8.5)	8.88, d, (9.3)	8.89, d, (9.2)	6.45, d, (8.0)
8	4.57, d, (6.3)	4.18, d, (6.2)	4.59, d, (6.5)	4.17, d, (5.6)	4.59, d, (6.4)	4.60, d, (6.4)	4.17[f]
9	5.67, dt, (10.3, 6.3)	5.42, dt, (10.3, 6.2)	5.62, dt, (10.4, 6.5)	5.45, dt, (10.2, 5.6)	5.66, dt, (10.3, 6.4)	5.66, dt, (10.3, 6.4)	5.49, m
12	7.06, dd, (8.6, 2.6)	7.04, dd, (8.3, 2.5)	7.04, dd, (8.3, 2.4)	7.03, dd, (8.2, 2.5)	7.00, dd, (8.3, 2.6)	6.96, dd, (8.5, 2.4)	7.04, dd, (7.8, 2.3)
13	7.09[f]	6.96, dd, (8.3, 1.3)	7.13[f]	6.96, dd, (8.2, 1.8)	7.11[f]	7.07[f]	6.97, dd, (7.8, 1.3)
15	7.10[f]	7.07, dd, (8.7, 1.3)	7.09[f]	7.06, dd, (8.5, 1.8)	7.11[f]	7.09[f]	7.07, dd, (7.5, 1.3)
16	7.48, dd, (8.2, 2.6)	7.15−7.31[f]	7.46, dd, (8.4, 2.4)	7.25[f]	7.46, dd, (8.7, 2.6)	7.46[f]	7.26[f]
17a	3.25, dd, (13.7, 8.4)	2.78, dd, (14.1, 5.4)	3.27, dd, (13.7, 7.6)	2.71, dd, (14.2, 5.5)	3.20, dd, (13.7, 7.8)	3.16, dd, (13.5, 7.6)	2.64, dd, (14.2, 5.1)
17b	3.30, dd, (13.7, 4.5)	3.25, dd, (14.1, 4.6)	3.32, dd, (13.7, 4.6)	3.26, dd, (14.2, 4.3)	3.28, dd, (13.7, 4.3)	3.29, dd, (13.5, 4.2)	3.40, dd, (14.2, 4.2)
19	7.44, d, (7.6)	7.15−7.31[f]	7.43, d, (7.4)	7.25[f]	7.43, d, (7.5)	7.45, d, (7.5)	7.29[f]
20	7.11, t, (7.6)	7.15−7.31[f]	7.15, t, (7.4)	7.27[f]	7.14, t, (7.5)	7.13, t, (7.5)	7.35, t, (7.5)
21	7.04, t, (7.6)	7.15−7.31[f]	7.07[f]	7.21[f]	7.05, t, (7.5)	7.03, t, (7.5)	7.27[f]
22	7.11, t, (7.6)	7.15−7.31[f]	7.15, t, (7.4)	7.27[f]	7.14, t, (7.5)	7.13, t, (7.5)	7.35, t, (7.5)
23	7.43, d, (7.6)	7.15−7.31[f]	7.43, d, (7.4)	7.25[f]	7.43, d, (7.5)	7.45, d, (7.5)	7.29[f]
24a	2.16, m	2.28, m	2.09, m	2.20, m	2.20, m	2.17, m	2.19, m
24b	2.47, m	2.62, m	2.39, m	2.59, m	2.47, m	2.45, m	2.59, m
25a	3.69, dt, (10.5, 5.7)	3.54, m	3.67, ddd, (12.4, 10.0, 5.7)	3.48, m	3.65, m	3.63, m	3.48, m
25b	4.33, t, (10.5)	4.57, m	4.46, t, (10.0)	4.17, dd, (12.3, 6.8)	4.36, t, (9.5)	4.36, t, (9.4)	4.17, m
28	5.01, t, (7.9)	4.41, t, (8.5)	4.85, d, (10.9)	4.41, d, (7.5)	4.73, t, (7.2)	4.69, t, (7.3)	4.53, t, (8.1)
29	2.27, m	1.99, m	2.36, m	1.99, dq, (13.5, 6.7)	2.24, m	2.20, m	1.92, m
30	1.04, d, (6.7)	0.95, d, (6.6)	0.97, d, (6.6)	0.91, d, (6.7)	1.06, d, (6.7)	1.03, d, (6.7)	0.81, d, (6.8)
31	0.98, d, (6.7)	0.77, d, (6.6)	0.91, d, (6.6)	0.81, d, (6.7)	1.04, d, (6.7)	1.02, d, (6.7)	0.80, d, (6.8)
32	8.60, d, (7.9)			8.37, d, (7.5)	11.0, d, (7.2)	11.86, d, (7.3)	6.80, d, (8.1)
34			2.86, dq, (6.5, 1.6)	4.24, q, (6.6)	4.65, q, (6.7)	4.26, q, (6.7)	5.04, q, (7.1)
35	2.35, s	2.09, s	1.37, d, (6.5)	1.57, d, (6.6)	1.78, d, (6.7)	1.78, d, (6.7)	1.41, d, (7.1)
37	14.20, s	11.05, s	2.32, s	2.90, s	3.68, s	3.66, s	8.17, s
38			4.55, d, (5.5)	2.90, s	3.51, s	3.29, s	2.96, s
			4.05, dd, (5.5, 1.6)				

[a]Recorded at 600 MHz. [b]Recorded at 500 MHz. [c]Recorded at 400 MHz. [d]Recorded in pyridine-d_5. [e]Recorded in chloroform-d_1. [f]Overlapped.

detection at 215, 230, 254, and 280 nm) eluting with 70% CH_3CN that contained 0.4 ‰ TFA to get **1** (20.0 mg), **2** (30.3 mg), **3** (70.2 mg), and **6** (7.2 mg). Fraction VI (24 g) was subjected to CC on silica gel ($CHCl_3$/$(CH_3)_2CO$, 5:1), Sephadex LH-20 (MeOH), and Lichroprep RP-18 gel (MeOH/H_2O, 50:50−70:30) and further purified by HPLC (Zorbax Eclipse-C_{18}, 5 μM, 9.4 mm × 250 mm, 1.0 mL/min, UV detection at 215, 230, 254, and 280 nm) eluting with 60% CH_3CN that contained 0.4 ‰ TFA to provide **4** (120.2 mg) and **5** (60.2 mg).

Air-dried and powdered roots (32.0 kg) were extracted three times with MeOH and treated according to the above acid−base method to obtain the alkaloid extract (20 g). The alkaloid extract was loaded onto a silica gel column and eluted with increasingly polar $CHCl_3$/MeOH. Four major fractions (I−IV) were provided, and fraction III was subjected to silica gel CC ($CHCl_3$/$(CH_3)_2CO$, 5:1) to get **7** (12.0 g) and then Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) and Lichroprep RP-18 gel (MeOH/H_2O, 85%) to obtain **5** (9.0 mg) and **8** (6.1 mg).

*Apetaline A (**1**):* white powder; mp 162−163 °C; $[\alpha]^{22}_D$ −19.3 (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.55) nm; IR (KBr) ν_{max} 3424, 2959, 2925, 1676, 1628, 1509, 1440, 1210, 1186 cm^{-1}; CD (*c* 0.13, MeOH) ($\Delta\varepsilon$) 197 (−4.88), 223 (−1.88), 232 (−2.21) nm; ^{1}H NMR (C_5D_5N, 600 MHz; $CDCl_3$, 400 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz; $CDCl_3$, 100 MHz) spectroscopic data (Tables 1 and 2); positive ESIMS *m*/*z* 584 $[M + Na]^+$ (30), 446 (30), 378 (10); positive HRESIMS, *m*/*z* 584.2481 $[M + Na]^+$ (calcd for $C_{30}H_{35}N_5O_6Na$, 584.2485).

*Apetaline B (**2**):* yellow powder; mp 149−150 °C; $[\alpha]^{16}_D$ −259.1 (*c* 0.50, MeOH); UV (MeOH) λ_{max} 204 (4.63), 309 (3.34) nm; IR (KBr) ν_{max} 3396, 2961, 2929, 1692, 1626, 1506, 1452, 1435, 1228 cm^{-1}; CD (*c* 0.13, MeOH) ($\Delta\varepsilon$) 199 (−19.94), 222 (−6.99), 231 (−8.33) nm; ^{1}H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz) spectroscopic data (Tables 1 and 2); positive FABMS, *m*/*z* 573 $[M]^+$ (10), 378 (2), 292 (2), 264 (5), 195 (12); positive HRESIMS, *m*/*z* 596.2858 $[M + Na]^+$ (calcd for $C_{32}H_{39}N_5O_5Na$, 596.2848).

*Epimauritine A (**3**):* white powder; mp 181−182 °C; $[\alpha]^{15}_D$ −231.1 (*c* 0.50, MeOH); UV (MeOH) λ_{max} 193 (4.25), 203 (4.65) nm; IR (KBr) ν_{max} 3394, 2964, 2929, 1677, 1508, 1453, 1438, 1205 cm^{-1}; CD (*c* 0.13, MeOH) ($\Delta\varepsilon$) 200 (−14.46), 221 (−2.36), 225 (−2.34), 229 (−3.15), 232 (−3.29) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) spectroscopic data (Tables 1 and 2); positive FABMS, *m*/*z* 576 $[M + H]^+$ (25), 292 (5), 221 (5); positive HRESIMS *m*/*z* 576.3186 $[M + H]^+$ (calcd for $C_{32}H_{42}N_5O_5$, 576.3185).

*Epimauritine A N-oxide (**4**):* white powder; mp 146−147 °C; $[\alpha]^{17}_D$ −1163.9 (*c* 0.10, MeOH); UV (MeOH) λ_{max} 204 (3.43), 309 (3.08) nm; IR (KBr) ν_{max} 3391, 2959, 2926, 1676, 1507, 1458, 1439,

dx.doi.org/10.1021/np200755t | J. Nat. Prod. 2011, 74, 2571−2575

1229 cm^{-1}; CD (*c* 0.018, MeOH) ($\Delta\varepsilon$) 200 (−33.72), 201 (−33.70), 223 (−5.44), 234 (−8.26) nm; ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectroscopic data (Tables 1 and 2); positive FABMS, *m/z* 592 $[M + H]^+$ (50), 378 (28), 203 (5); positive HRESIMS, *m/z* 592.3126 $[M + H]^+$ (calcd for $C_{32}H_{42}N_5O_6$, 592.3135).

*Mauritine A N-oxide (**5**):* yellow powder; mp 136−137 °C; $[\alpha]^{16}_D$ −376.6 (*c* 0.10, MeOH); UV (MeOH) λ_{max} 204 (5.08) nm; IR (KBr) ν_{max} 3390, 3057, 3031, 1671, 1506, 1453, 1434, 1228 cm^{-1}; CD (*c* 0.02, MeOH) ($\Delta\varepsilon$) 200 (−40.63), 223 (−8.65), 233 (−12.20) nm; ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz) spectroscopic data (Tables 1 and 2); positive FABMS *m/z* 592 $[M + H]^+$ (25), 378 (28), 203 (5); positive HRESIMS *m/z* 592.3122 $[M + H]^+$ (calcd for $C_{32}H_{42}N_5O_6$, 592.3135).

*Apetaline C (**6**):* white powder; mp 139−140 °C; $[\alpha]^{15}_D$ −29.8 (*c* 1.00, MeOH); UV (MeOH) λ_{max} 206 (0.78) nm; IR (KBr) ν_{max} 3426, 2958, 2928, 1676, 1508, 1457, 1384, 1203 cm^{-1}; CD (*c* 0.30, MeOH) ($\Delta\varepsilon$) 200 (−10.39), 222 (−2.91), 224 (−2.76), 232 (−3.43), 262 (0.12) nm; ^1H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz) spectroscopic data (Tables 1 and 2); positive ESIMS *m/z* 612 $[M + Na]^+$ (5); positive HRESIMS *m/z* 612.2782 $[M + Na]^+$ (calcd for $C_{32}H_{39}N_5O_6Na$, 612.2798).

*Mauritine A (**7**):* white powder; CD nm ($\Delta\varepsilon$) 200 (−20.23), 223 (−4.0), 234 (−5.81) nm; ^1H NMR ($CDCl_3/C_5D_5N$, 400/500 MHz) and ^{13}C NMR ($CDCl_3/C_5D_5N$, 100/125 MHz) spectroscopic data (Supporting Information); positive ESIMS *m/z* 576 $[M + H]^+$ (20), 378 (5), 199 (2).

*Mauritine F (**8**):* white powder; ^1H NMR ($CDCl_3/C_5D_5N$, 400/500 MHz) and ^{13}C NMR ($CDCl_3/C_5D_5N$, 100/125 MHz) spectroscopic data (Supporting Information); positive ESIMS *m/z* 562 $[M + H]^+$ (10), 378 (5).

Configuration of 1−8 (Marfey's Method[7]). Compounds **1**−**8** (1 mg each) were separately dissolved in 6 N HCl (1 mL) in a sealed glass tube and incubated at 115 °C for 24 h. The hydrolysate was dried under a stream of N_2 to remove the remaining HCl. The resultant hydrolysate was dissolved in 0.9 mL of acetone, and 1 M $NaHCO_3$ (20 μL) and 1% Nα-(2,4-dinitro-5-fluorophenyl)-L-alaninamide (L-FDAA, Marfey's reagent, Sigma Aldrich, 100 μL) were added. The mixture was incubated at 40 °C for 1 h. The reaction was quenched by adding 2 N HCl (10 μL) after being cooled, and the dried mixture was dissolved in 50% aqueous CH_3CN (600 μL) to yield FDDA derivatives. The standard amino acids D-Phe, L-Phe, D-Val, L-Val, D-*N*-methylalanine, L-*N*-methylalanine, D-*N*,*N*-dimethylalanine, and L-*N*,*N*-dimethylalanine were also treated with the above method. A 5 μL amount of the FDAA derivate was analyzed by HPLC with a RP-18 column (Agilent, Zorbax Eclipse-C_{18}, 5 μM, 4.6 mm × 150 mm). The column temperature was maintained at 30 °C. Mobile phase A was H_2O with 0.4 ‰ TFA, and mobile phase B was CH_3CN. The gradient was 0−40 min, 10−25%; 40−41 min, 25−30%; 41−60 min, 30−40%. The flow rate was 1 mL/min. FDAA-derivate amino acids were detected at 254 and 340 nm, compared with the retention times of standard amino acids as follows: 55.81 min (L-Phe), 30.02 min (D-Phe); 44.51 min (L-Val), 62.73 min (D-Val); 33.21 min (L-N(CH_3)-Ala), 48.25 min (D-N(CH_3)-Ala); and 38.57 min (L-N,N(CH_3)$_2$-Ala), 27.55 min (D-N,N(CH_3)$_2$-Ala).

11β-HSD1 Inhibitory Assays. The inhibitory activities of mauritine A (**7**) on human or murine 11β-HSD1 and 11β-HSD2 were analyzed using the scintillation proximity assay (SPA). Microsomes containing 11β-HSD1 or 11β-HSD2 were used according to our previous studies.[14] The full-length cDNAs of human or mouse 11β-HSD1 and 11β-HSD2 were isolated from the cDNA libraries provided by NIH Mammalian Gene Collection. The cDNAs were cloned in pcDNA3 expression plasmid. HEK-293 cells were transformed with the pcDNA3-derived expression plasmid and screened by 700 μg/mL G418. The microsomal fraction containing 11β-HSD1 or 11β-HSD2 was prepared from the HEK-293 cells, which stably express 11β-HSD1 or 11β-HSD2, and then was used for SPA. Briefly, microsomes were incubated with NADPH and $[^3H]$ cortisone. The product, $[^3H]$ cortisol, was specifically captured by monoclonal antibody coupling to protein A-coated SPA beads. The 11β-HSD2 screening was performed by incubating 11β-HSD2 microsomes with $[^3H]$ cortisol and NAD$^+$ and monitoring substrate disappearance. Glycyrrhizinic acid was set as a positive control. IC_{50} (X ± SD, *n* = 3) values were calculated by using Prism Version 4 (GraphPad Software, San Diego, CA, USA). The IC_{50} values of glycyrrhizinic acid (positive control) were 29.5, 18.6, and 0.7 nM for murine 11β-HSD1, human 11β-HSD1, and human 11β-HSD2, respectively.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

1D and 2D NMR spectral data of compounds **1**−**8** are available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +86-871-5223800. Fax: +86-871-5223800. E-mail: nhtan@mail.kib.ac.cn.

Author Contributions

‡Contributed equally to this work.

■ ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (30725048, 91013002, U1032602), the National New Drug Innovation Major Project of China (2011ZX09307-002-02), the National Basic Research Program of China (2009CB522300), and the Fund of Chinese Academy of Sciences (KSCX2-YW-R-177). The authors are grateful to the members of the analytical group from the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, for measuring optical rotations and for the IR, UV, CD, NMR, and mass spectra.

■ REFERENCES

(1) National Pharmacopoeia Committee of China. *Chinese Pharmacopoeia*; Chinese Medical Science Press: Bejing, 2010; pp 343−344.

(2) Tan, N. H.; Zhou, J. *Chem. Rev.* **2006**, *106*, 840−895.

(3) Ménard, E. L.; Müller, J. M.; Thomas, A. F.; Bhatnagar, S. S.; Dastoor, N. J. *Helv. Chim. Acta* **1963**, *46*, 1801−1811.

(4) El-Seedi, H.; Zahra, M.; Goransson, U.; Verpoorte, R. *Phytochem. Rev.* **2007**, *6*, 143−165.

(5) Cristau, P.; Temal-Laïb, T.; Bois-Choussy, M.; Martin, M.-T.; Vors, J.-P.; Zhu, J. *Chem.—Eur. J.* **2005**, *11*, 2668−2679.

(6) Will, A. K. A. G. *Z. Naturforsch.* **1976**, *31b*, 279−280.

(7) Marfey, P.; Ottesen, M. *Carl. Res. Commun.* **1984**, *49*, 585−590.

(8) Catherine, F. L. *J. Nat. Prod.* **1996**, *59*, 676−678.

(9) Shah, A. H.; Pandey, V. B.; Eckhardt, G.; Tschesche, R. *Phytochemistry* **1985**, *24*, 2765−2767.

(10) El-Seedi, H. R.; Gohil, S.; Perera, P.; Torssell, K. B. G.; Bohlin, L. *Phytochemistry* **1999**, *52*, 1739−1744.

(11) Singh, B.; Pandey, V. B. *Phytochemistry* **1995**, *38*, 271−273.

(12) Gournelis, D. C.; Laskaris, G. G.; Verpoorte, R. In *Cylopeptide Alkaloids*; Herz, W., Falk, H., Kirby, G. W., Moore, R. E., Tamm, C. H., Eds.; Progress in the Chemistry of Organic Natural Products; Springer: New York, 1998; pp 1−179.

(13) Zhou, J.; Tan, N. H. *Chin. Sci. Bull.* **2000**, *45*, 1825−1831.

(14) Yang, H.; Dou, W.; Lou, J.; Leng, Y.; Shen, J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1340−1345.