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Antiplasmodial and antimycobacterial cyclopeptide alkaloids from the root of *Ziziphus mauritiana*

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ABSTRACT

Investigation of the MeOH extract obtained from the root of the *Ziziphus mauritiana* grown in Thailand resulted in the isolation of two 14- and 13-membered cyclic alkaloids, mauritine L (**1**) and mauritine M (**2**), and three known cyclopeptide alkaloids, nummularines H (**3**), B (**4**) and hemsine A (**5**). Their structures were elucidated on the basis of extensive NMR spectroscopic analysis. The first single crystal X-ray diffraction study of the 13-membered ring cyclopeptide, nummularine B methiodide (**4′**), revealed all *S* configurations on the amino acid residues. The isolated alkaloids exhibited potent antiplasmodial activity against the parasite *Plasmodium falciparum* with the inhibitory concentration (IC_{50}) ranging from 3.7 to 10.3 μM. Compounds **2** and **3** also demonstrated antimycobacterial activity against *Mycobacterium tuberculosis* with the MIC of 72.8 and 4.5 μM, respectively.

1. Introduction

Phytochemical investigations have established the genus *Ziziphus* (Rhamnaceae family) to be a rich source of cyclopeptide alkaloids (Hesham et al., 2007; Tan and Zhou, 2006), lupane and ceanothane triterpenes (Suksamrarn et al., 2006). Cyclopeptide macrocycles of *Ziziphus* species showed interesting biological properties, including, for example, sedative (Han et al., 1989), analgesic (Trevisan et al., 2009), antibacterial (Morel et al., 2002), antifungal (Pandy and Devi, 1990), antiplasmodial (Suksamrarn et al., 2005) and immunostimulant (Lin et al., 2000) activities. *Ziziphus mauritiana* Lam. or Phut-sa in Thai is a medium-sized tree, native to Thailand and Asian countries and has been used traditionally for treatment of diarrhea, ulcers, vomiting and indigestion (Bunyaprapatsara and Chokechaijaroenporn, 1999). In continuation of our work on new antimalarial substance of new structural type from *Ziziphus*, the root extract of *Z. mauritiana* was screened and exhibited *in vitro* antiplasmodial potential against *Plasmodium falciparum*. Previous phytochemical studies of this plant species established the 14-membered ring cyclopeptides to be the largest subgroup of alkaloid obtained, whereas only one 13-membered macrocyclic alkaloid isolated from this plant (Gournelis et al., 1998). These included the 4(14)-membered ring class: mauritine C, amphibine F and frangufoline (Tschesche et al., 1974b); the 5(14)-membered ring type: mauritines A and B (Tschesche et al., 1972), D–F (Tschesche et al., 1974b), H (Tschesche et al., 1977), J (Jossang et al., 1996), K (Singh et al., 2007), and amphibines B and E (Tschesche et al., 1974b), D (Tschesche et al., 1972), and the 4(13)-membered cyclic alkaloid sativanine K (Singh et al., 2007). The low natural abundance together with the interesting scaffold of cyclopeptides has attracted several synthetic groups to increase their availability (Schmidt et al., 1983; Heffner et al., 1992; Gournelis et al., 1998; Temal-Laïb et al., 2002; He et al., 2007; Toumi et al., 2007a, 2007b, 2008), including mauritines A–C, F (Laib et al., 2000; Cristau et al., 2005), mauritine D and amphibine E and their epimers (Kim et al., 2003; Joullie and Richard, 2004) and frangufoline (Xiao et al., 1998). In this paper, the isolation and structure elucidation of two new cyclopeptide alkaloids of the 4(14)-type, mauritine L (**1**), and the 5(13)-type, mauritine M (**2**), are described together with three known alkaloids nummularines H and B (**3–4**) and hemsine A (**5**) from the root of *Z. mauritiana*. Antimalarial and antimycobacterial evaluations of the isolates are also reported here (Fig. 1).

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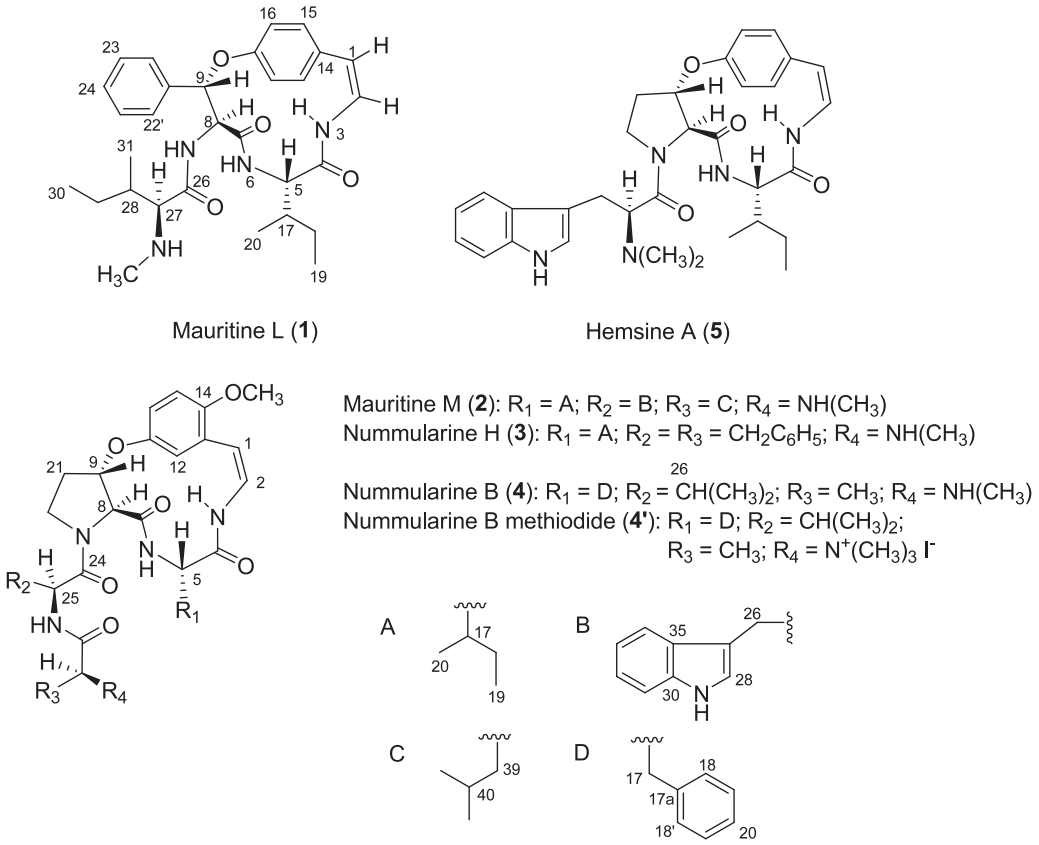

Fig. 1. Chemical structures of compounds **1–5**.

2. Results and discussion

The pulverized, dried root of *Z. mauritiana* was extracted successively with EtOAc and MeOH. The resulting extracts were tested for antimalarial and antituberculosis activities and only the MeOH extract was active to the antiplasmodial test. A typical intense blue coloration with anisaldehyde–H_2SO_4 reagent for the MeOH extract indicated the presence of cyclopeptide alkaloid (Suksamrarn et al., 2005). However, the EtOAc extract gave very weak blue color development. The MeOH soluble extract was therefore selected for further chromatographic separations and resulted in the isolation of two new, in addition to three known, cyclopeptide alkaloids. The UV absorption bands at around 270 and 320 nm were observed for the 13-membered cyclopeptides **2–4**, which is the characteristic styrylamine chromophore. Due to the strain of the ring system in the molecule, the 14-membered cyclopeptide alkaloid lacked these absorption bands, except for the presence of tryptophan moiety, which showed the absorption bands at around 220, 270 and 290 nm (Gournelis et al., 1998). Their IR spectra exhibited diagnostic peaks for amino (3291–3393 cm^{-1}), amide (1679–1693 cm^{-1}), and aryl ether (1221–1237 cm^{-1}) functions.

Compound **1** was isolated as a colorless powder and the molecular formula $C_{30}H_{40}N_4O_4$ was established by HRTOFMS at *m/z* 521.3124 $[M+H]^+$. The ^{13}C NMR and DEPT spectra ($CDCl_3$, 75 MHz) indicated 30 signals attributable to four methyls, one *N*-methyl, two methylenes, seventeen (including one oxygenated and two olefinic) methines and six quaternary carbons, three of which corresponded to the carbonyl groups (Table 1). The ^1H NMR spectrum of **1** (Table 1) displayed signals corresponding to *Z*-olefinic protons of styrylamine at δ_H 6.36 (*d*, *J* = 7.3 Hz) and 6.71 (*dd*, *J* = 9.7, 7.3 Hz), a number of aromatic, methine, methylene and methyl protons including a singlet of *N*-methyl proton at δ_H 2.05. From the ^1H–^1H COSY and ^1H–^{13}C HMQC spectra of **1** and comparison with the reported values led to a conclusion for the presence of *p*-oxystyrylamine group, *β*-phenylserine, isoleucine and *N*-methylisoleucine amino acid units (Tan and Zhou, 2006). Analysis of the COSY, HMBC and NOESY spectra provided the connections among these subunits (Fig. 1). The signal for *p*-oxystyrylamino NH-3 (δ_H 6.57) showed weak NOESY correlation to signal at δ_H 4.03 (H-5) and the HMBC correlations of the resonance at δ_H 6.71 (H-2) to C-14 and of H-1 (δ_H 6.36) to C-2 allowed the placement of an isoleucine moiety next to the styrylamine group. The correlations of H-8 (δ_H 4.64, *dd*, *J* = 8.4, 6.3 Hz) to NH-6 (δ_H 6.42, *d*, *J* = 7.8 Hz) and H-22′ (δ_H 7.50) in the NOESY and of H-9 (δ_H 6.17, *d*, *J* = 6.3 Hz) to C-7 (δ_C 171.5), C-21 (δ_C 137.2) and C-22 (δ_C 127.5) in the HMBC experiments established the connection of isoleucine to *β*-phenylserine fragments in the macrocyclic ring. The NOESY cross-peak for methine proton H-9 to aromatic signal at δ_H 7.34 (H-12) was also observed. The *β*-phenylserine moiety was characterized as *erythro* relative configuration by the vicinal coupling constant (*J* = 6.3 Hz) between H-8 and H-9 and the relatively upfield shift of the ^{13}C NMR signal of C-9 (δ_C 81.4) compared with δ_C 86.3 of condaline A for the *threo* form (Morel et al., 2005). NOE enhancements displayed between NH-25 to H-8 and H-27 in the NOESY spectrum, and the HMBC of the latter with the carbonyl carbon signal at δ_C 173.5 (C-26) supported that the *N*-methylisoleucine unit was attached to the phenylserine at N-25. HMBC correlations of the methyl proton signals at δ_H 0.62 (H-31) and δ_H 2.05 (N-CH_3) to the signal at δ_C 69.6 (C-27) were also observed (Fig. 2).

The CD spectrum of **1** displayed an intense negative and a weak positive Cotton effect bands at 239 and 279 nm, respectively, consistent with the 5*S*,8*S*,9*S*-configurations presented in the 14-membered ring nucleus (Gournelis et al., 1998). In addition, the NMR spectroscopic data of the macrocyclic part of **1** are in good

Table 1
^{1}H and ^{13}C NMR spectroscopic data for compounds **1**, **2**, **4** and **4′**.[a]

Position	δ_H				δ_C			
	1[b]	**2**[b]	**4**[b]	**4′**[c]	**1**[b]	**2**[b]	**4**[b]	**4′**[c]
1	6.36 *d* (7.3)	5.91 *d* (9.0)	5.95 *d* (8.9)	6.11 *d* (8.9)	115.5	107.2	107.2	110.7
2	6.71 *dd* (9.7, 7.3)	6.91 *dd* (11.2, 9.0)	6.94 *dd* (11.0, 8.9)	6.87 *d* (8.9)	125.5	121.3	121.4	121.8
3-NH	6.57 *br d* (9.7)	8.35 *d* (11.2)	8.41 *d* (11.0)		–			
4					167.1	167.2	167.1	170.4
5	4.03 *dd* (7.8, 3.1)	4.23 *t* (4.5)4.56 *m* [d]	4.56 *m*	4.51*dd* (10.9, 3.6)	59.6	60.5	56.7	58.7
6-NH	6.42 *d* (7.8)	7.25[d]		7.30 *m* [d]				
7			–		171.5	170.2	170.1	172.3
8	4.64 *dd* (8.4, 6.3)	4.38 *d* (3.2)	4.43 *br s*	4.49 *d* (3.0)	56.4	64.3	64.2	66.1
9	6.17 *d* (6.3)	5.31 *dt* (7.2, 3.2)	5.49 *br s*	5.34 *dt* (7.7, 3.0)	81.4	76.4	76.5	78.9
11			–		155.1	150.9	151.0	152.4
12	7.34 *m* [d]	6.61 *d* (2.9)	6.67 *br s*	6.84 *br s*	123.5	111.2	111.3	112.6
13	7.12 *br t* (7.8)				130.1 [e]	124.1	124.1	125.3
14					132.2	151.4	151.5	153.1
15	7.12 *br t* (7.8)	6.84 *d* (9.0)	6.87 *d* (9.0)	7.04 *d* (9.0)	132.2 [e]	113.7	113.8	115.2
16	7.34 *m* [d]	6.74 [d]	6.73 *br d* (9.0)	6.89 *dd* (9.0, 2.8)	123.6	117.6	117.8	119.0
17	2.15 *m*	2.00 *m*	3.25 *dd* (14.0, 3.3)	3.21 *dd* (14.0, 3.6)	35.0	35.4	36.6	37.5
			2.86 *dd* (14.0, 10.0)	2.87 *dd* (14.0, 10.9)				
17a							135.7	138.2
18	1.61and 0.95	1.43 and 1.15				24.0	24.6	
(each *m*)		(each *m*)						
18, 18′			7.21–7.32 *m*	7.29 *m*			129.2	130.0
19	0.81 *t* (7.2)	0.87 *t* (7.3)					12.1	11.7
19, 19′					7.21–7.32 *m*	7.29 *m*	128.8	129.6
20	0.66 *d* (6.4)	0.96 *d* (6.9)	7.21–7.32 *m*	7.23 *m*	16.0	16.0	127.2	127.8
21								137.2
21α			2.08 *m*	2.27 *m*	2.28 *m*	32.3	32.5	33.7
21β		2.25 *m*		2.54 *m*		2.61 *m*		
22, 22′	7.50 *br d* (7.4)				127.5			
22α		3.79 *m*[d]	4.11 *br t* (8.7)	4.27 *m*		46.2	46.5	47.7
22β	2.46 *m*		3.47 *m*		3.60 *m*			
23, 23′	7.40 *m* [d]		–				128.9	
24	7.40 *m* [d]		–		128.7	171.3	171.3	171.6
25		5.09 *dt* (8.4, 5.1)	4.51 *m*		4.37 *d* (8.3)	50.3	54.2	57.9
25-NH	7.43[d]							
26		3.21 *dd* (14.0, 5.1)	1.77 *hept* (6.9)	1.90 *hept* (7.0)	173.5	29.3	30.9	31.8
		3.02 *dd* (14.0, 8.4)						
27	2.52 *d* (4.1)		0.57 *d* (6.3)	0.62 *d* (6.6)	69.6	109.3	17.2	19.0
28	1.36 *m*	6.76 [d]	0.68 *d* (6.3)	0.96 *d* (6.6)	37.5	122.9	19.1	19.2
29	0.75 and 0.51 (each *m*)					23.7		
29-NH			8.58 *br s*		7.59 *d* (8.7)			
30	0.65 *t* (6.4)				11.7	136.0	174.9	168.7
31	0.62 *d* (6.8)	7.31 *d* (7.8)	3.07 *br q* (6.6)	4.22 *q* (6.5)	15.7	111.4	60.5	71.2
32			7.15 *br t* (7.6)	1.32 *d* (6.6)	1.65 *d* (6.7)	122.1	19.8	13.2
33			7.09 *br t* (7.3)			119.6		
34			7.65 *d* (7.5)			118.4		
35							127.2	
36-NH			7.86 *d* (8.0)					
37						174.0		
38	3.06 *br t* (8.5)						63.1	
39			1.56 and 1.41 (each *m*)			42.3		
40			1.63 *m*				25.0	
41			0.90 *d* (7.0)			21.9		
42			0.92 *d* (7.0)				23.0	
OMe		3.75 *s*	3.78 *s*		3.82 *s*	55.9	56.0	56.7
NMe	2.05 *s*	2.37 *s*	2.42 *s*		36.5	35.0	35.1	
$N^+(Me)_3$					3.30 *s*			52.7

[a] The coupling constants (*J*) are in Hz in parentheses; chemical shifts are given in ppm.
[b] NMR data in $CDCl_3$.
[c] NMR data in CD_3OD.
[d] Signals without multiplicity was assigned from COSY.
[e] Signals under the same superscript may be reversed.

agreement with those of previously studied scutianine M, a structurally related alkaloid isolated from *Scutia buxifolia* which was composed of the same sub-units, where the L-amino acids were determined by analysis of the hydrolyzed residues and the *J* coupling value (Morel et al., 2005). The ^{13}C NMR spectroscopic data of the terminal *N*-methylisoleucine unit was similar to that of the synthesized model dipeptide, L-Ile(OMe)-L-Ile(NMe_2) and of the *N*-methylisoleucine in paliurine B (Lin et al., 2000; Lee et al., 2001), suggesting the configuration of the acyclic amino acid residue attached to the macrocyclic ring at N-25 of **1** was *L*. The stereochemistry of **1** was therefore deduced as shown. Thus the structure of **1** was established as the *N*-desmethyl analogue of nummularine M, the 4(14)-integerrine-type cyclopeptide alkaloid, named mauritine L after its plant origin.

Mauritine M (**2**) was obtained as a colorless solid, mp 188–189 °C. On the basis of its HRTOFMS (*m/z* 687.3856, $[M+H]^+$) in combination with analysis of the ^{13}C NMR spectrum, the molecular formula of **2** was established as $C_{38}H_{50}N_6O_6$. The UV absorption

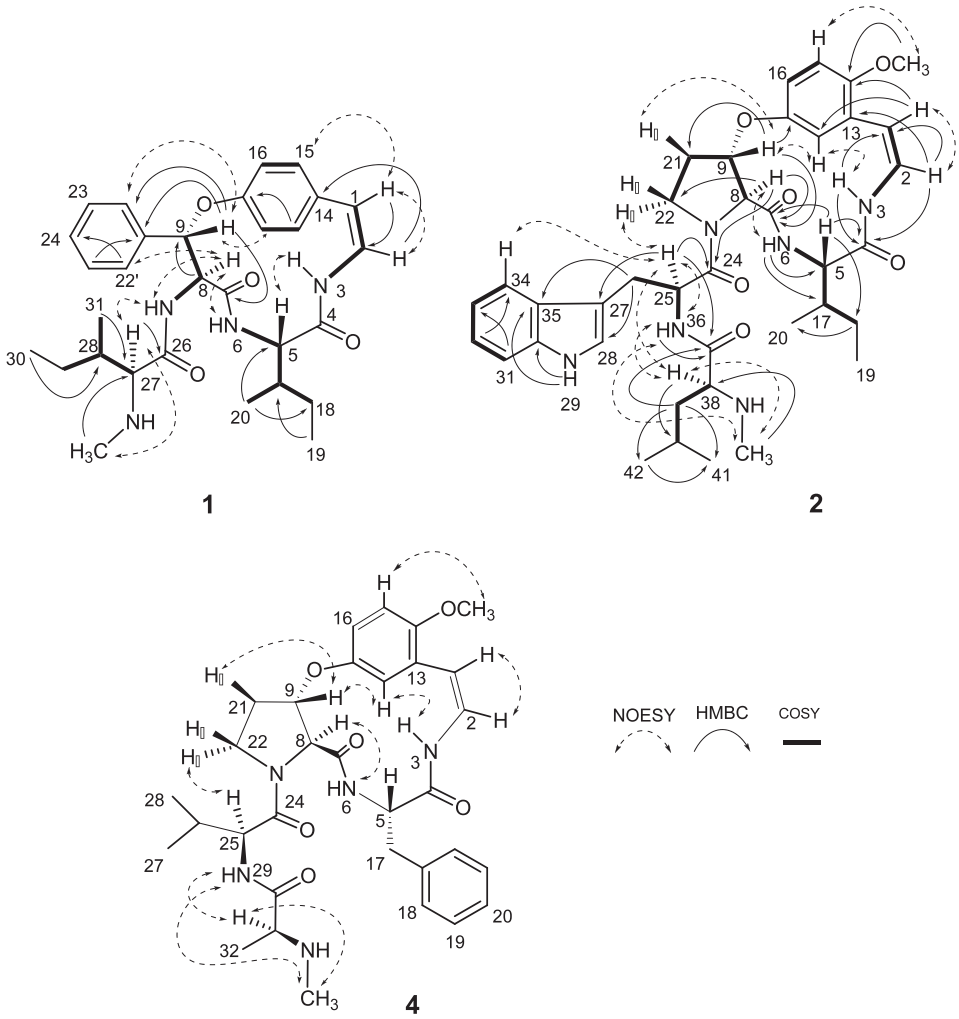

Fig. 2. Selected COSY, HMBC and NOESY interactions for compounds **1–2** and **4**.

maxima at 219, 272, 289 and 318 nm of **2** indicated the presence of a tryptophan moiety of 13-membered zizyphine A-typed cyclopeptide alkaloid (Gournelis et al., 1998). The ^{1}H NMR spectrum (Table 1) displayed signals for a *Z* double bond at δ 5.91 (*d*, *J* = 9.0 Hz) and 6.91 (*dd*, *J* = 11.2, 9.0 Hz), a singlet methoxyl (δ 3.75), a singlet *N*-methyl (δ_H 2.37), three methyl doublets, one methyl triplet, a number of aromatic, methine and methylene protons, including NH signals at δ_H 8.58 (br *s*), 8.35 (*d*, *J* = 11.2 Hz) and 7.86 (*d*, *J* = 8.0 Hz). The ^{13}C NMR and DEPT spectra of **2** showed 38 carbon resonances for four methyls (δ_C 11.7, 16.0, 21.9 and 23.0), one methoxy (δ_C 55.9), one *N*-methyl (δ_C 35.0), five methylenes (δ_C 24.6, 29.3, 32.3, 42.3 and 46.2), seventeen methines (two of which were olefinic carbons at δ_C 107.2 and 121.3) and six quaternary aromatic carbons (δ_C 109.3, 111.4, 124.1, 127.2, 150.9 and 151.4), including four carbonyl carbons (δ_C 167.2, 170.2, 171.3 and 174.0).

Evidence for the spin systems of isoleucine, 3-oxygenated proline, tryptophan and *N*-methyleucine residues and a *m*-oxygenated *Z*-styrylamine group (Fig. 1) was obtained from the analysis of the ^{1}H, ^{13}C, DEPT and 2D NMR (COSY and HMQC) spectra of **2** (Table 1) in addition to the comparisons with the reported data. An intense peak at *m/z* 100 in the EIMS spectrum of **2** indicated an *N*-methyl leucine unit as the terminal amino acid. The resonance of styrylamino group at δ_H 8.35 (NH-3) and 6.91 (H-2) showed HMBC cross-peaks with the signals of isoleucyl carbonyl C-4 (δ_C 167.2). Correlations of H-2 to C-1 and C-13, and of H-1 to C-12 and C-14 were also observed. The triplet methine proton at δ_H 4.23 (H-5) exhibited HMBC cross peaks with C-4 and C-7 (δ_C 170.2) and in turn the latter showed a cross peak to the resonance at δ_H 4.38 (H-8) and 5.31 (H-9) indicating the connection between the isoleucine and β-hydroxyproline units. HMBC correlation from H-9 to C-11 confirmed the placement of the β-hydroxyproline next to the aryl group. The tryptophan moiety was identified by the cross-peaks shown between the methine hydrogen at δ_H 5.09 (H-25) and signals at δ_H 7.65 (H-34) and 7.86 (NH-36) in the NOESY spectrum. Additional HMBC correlations of H-25 with C-27 (δ_C 109.3) and of the diastereotopic protons H_2-26 (δ_H 3.21 and 3.02) with C-28 (δ_C 122.9) and C-35 (δ_C 127.2) were also observed. NOE enhancements observed from H-25 to H-22α, NH-36 and H-38 and from NH-36 to H-38 and *N*-methyl signal at δ_H 2.37 in the NOESY experiments, along with the ^{1}H–^{1}H COSY correlation of H-25 with NH-36, in addition to the HMBC correlations from H-8 signal to the carbonyl carbon (C-24, δ_C 171.3) and from H-25 to C-37 (δ_C 174.0) established the position of the tryptophan residue as the intermediate amino acid unit attached between the proline at N-23 and the terminal *N*-methylleucine group of the macrocyclic system. Thus, the structure of mauritine M (**2**) was established as the *N*-methylleucine analogue of nummularine R or daechuine S10, the NMR data of the latter of which has not been reported.

The known compounds **3–5**, by examination of their MS, 1D and 2D NMR spectra and comparison with the reported values, were identified to be of the zizyphine A-type alkaloids, nummularine H (Lee et al., 2001) and nummularine B (Tschesche et al., 1974a) and the amphibine B-typed, hemsine A (Lin et al., 2003), respec-

tively. Complete ^{1}H and ^{13}C NMR spectroscopic data assignments for **4** were recorded herein for the first time (Table 1). The X-ray diffraction analysis from crystals of **4′**, using anomalous signals from the iodide ion, confirmed that the skeleton was made up of a phenylalanine, a unit of hydroxyproline bearing a valine and *N*-methylalanine and a *Z*-styrylamine moieties. The X-ray analysis also provided the unambiguous absolute configuration assignments as 5*S*, 8*S*, 9*S*, 25*S* and 31*S* at the amino acid residues, as well as the endocyclic NH-3, H-9 and H-12 displayed in the macrocyclic ring (Fig. 3, see Supplementary material section). The refined Flack parameter was −0.01(3). This is the first X-ray crystal structure reported for the 13-membered zizyphine A-typed cyclopeptide alkaloid, though the quaternary ammonium salt might possess a slightly different feature from its parent compound.

Rhamnaceous cyclopeptide alkaloids, including zizyphine A-type alkaloids, are generally composed of L-amino acids and *trans*-*β*-hydroxy-L-proline. The CD spectrum of **2** exhibited three negative Cotton effect bands at 320, 263 and 209 nm and a small positive one at 228 nm which was similar to those of nummularines H (**3**) (Lee et al., 2001) and B (**4**) and was consistent with the 5*S*,8*S*,9*S*-configurations reported in the 13-membered ring macrocycle (Gournelis et al., 1998). The NOESY experiments associated with the value of coupling constants permitted the relative stereochemical determinations for **2–4** (Fig. 2). No significant NOE enhancements were observed for H-5/NH-6 and for H-8/H-9 in addition to a small vicinal coupling exhibited for H8/H-9 (J = 3.2–4.4 Hz), suggesting they were in the *trans* orientation (Suksamrarn et al., 2005) in **2–4**. Strong NOE effects between NH-3/H-12 and between H-12/H-9 displayed in **2–4** were consistent with the endocyclic arrangements among the three protons in the X-ray crystal structure for **4′**. Furthermore, the NOE enhancements observed for NH-6/H-8, H-22α/H-25 and H-38/*N*-methyl protons in the NOESY experiments of **2** were similar to those of the corresponding protons in compounds **3**, **4** and **4′** in their NOESY spectra, suggesting the same spatial orientations in their acyclic amino acid units. This evidence and their levorotatory optical rotations led to the conclusion that the cyclopeptides **2–4** share the same stereochemistry both at the ring nucleus and at the acyclic part as that of nummularine B methiodide (**4′**).

The *in vitro* antimalarial effect against *P. falciparum* (Trager and Jensen, 1976; Desjardins et al., 1979) of compounds **1–5** and nummularine B methiodide **4′** was evaluated. Mauritine M (**2**), nummularine H (**3**) and hemsine A (**5**) demonstrated potent antiplasmodial activity with the IC_{50} values of 3.7, 4.2 and 7.3 μM, respectively. Nummularine B (**4**) was moderately active (IC_{50} 10.3 μM) whereas compounds **1** and **4′** were inactive. The cyclopeptide **3** exhibited interesting antituberculosis activity against *Mycobacterium tuberculosis* (Collins and Franzblau, 1997) with the MIC value of 4.5 μM, whilst **2** showed weak activity (MIC 72.8 μM). Compounds **1**, **4**, **4′** and **5** were inactive to the same test. The relatively high antiplasmodial activity of **2**, **3** and **5** could possibly be due to the presence of hydroxyproline unit in the macrocycle ring and the terminal *N*-methylated or *N*,*N*-dimethylated amino acid residues. This is the first report of *in vitro* antimalarial and antituberculosis activities of 14-membered cyclopeptide alkaloids. Antiplasmodial and antimycobacterial potentials of the 13-membered ring macrocyclic alkaloids have previously been reported by our group (Suksamrarn et al., 2005).

3. Conclusion

Phytochemical investigation of *Z. mauritiana* root led to the isolation of two 4(14)-type and three 5(13)-type cyclopeptide alkaloids. This is the second report on the isolation of 13-membered ring cyclopeptides obtained from this plant species. *In vitro* antimalarial and antimycobacterial assays demonstrated that cyclopeptide alkaloids having a hydroxyproline and the terminal *N*-methylated or *N*,*N*-dimethylated amino acid residues displayed potent biological activities. Other *Ziziphus* plants are under investigation to confirm this finding.

4. Experimental

4.1. General

Optical rotations were taken on a JASCO-1020 digital polarimeter. Melting points were measured using a Griffin melting point

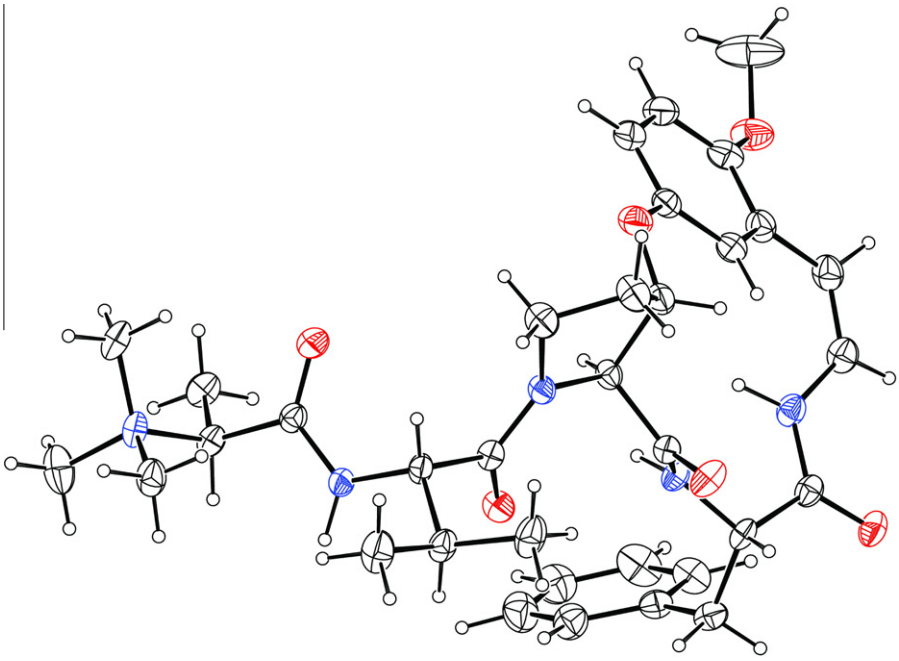

Fig. 3. ORTEP plot of the X-ray crystal structure for compound **4′**.

apparatus. UV and IR spectra were recorded on Shimadzu UV-2401 PC and Perkin Elmer FT-IR Spectrum BX spectrophotometers, respectively. The NMR spectra were recorded with a Bruker Avance 300 MHz spectrometer. For the spectra taken in $CDCl_3$ and CD_3OD, the residual nondeuterated solvent signals at δ 7.24 and δ 3.30 and the solvent signals at δ 77.0 and δ 49.0 were used as references for ^1H and ^{13}C NMR spectra, respectively. Mass spectra were recorded on a Thermo Finnigan LC-Q and a Bruker micrOTOF mass spectrometer. Open column chromatography was performed on Merck silica gel 60 (finer than 0.063 mm) and Sephadex LH-20. TLC was performed on Merck precoated silica gel plates (silica gel 60 F_{254} on aluminum foil) and spots on TLC were visualized under UV light and by spraying with anisaldehyde-H_2SO_4 followed by heating.

4.2. Materials and methods

Air-dried root of *Z. mauritiana* was collected from Samchuk District, Suphanburi Province, Thailand, in June 2005. A herbarium sample (Jessada Netsawangwicha 002) was identified by Nopporn Damrongsiri and has been deposited at the Faculty of Science, Ramkhamhaeng University, Thailand.

4.3. Extraction and separation

The pulverized, dry root (4.5 kg) of *Z. mauritiana* was extracted successively with EtOAc (3 × 10 L) followed by MeOH (3 × 10 L) at room temperature for each two weeks and the solvents were evaporated to yield the EtOAc (29.5 g) and MeOH (45.6 g) extracts, respectively. Both extracts were subjected to biological activity screenings and the MeOH extract exhibited antiplasmodial activity, whereas the EtOAc extract showed antimycobacterial potential. The MeOH extract gave a typical intense blue coloration with anisaldehyde–H_2SO_4 reagent on TLC but the EtOAc soluble extract gave very weak blue coloration. The MeOH soluble extract was thus selected for further extensive investigation by quick column chromatography (CC) (Pederson and Rosenbohm, 2001), eluted with a gradient of CH_2Cl_2–EtOAc, EtOAc, EtOAc–MeOH, MeOH and MeOH–H_2O (5–10% increment of the more polar component, each 300 mL) to provide six major fractions. The antiplasmodial active fraction 2 (5.23 g) was subjected to CC employing solvent gradient CH_2Cl_2–MeOH to obtain 11 subfractions (1–11). Subfraction 4 (895 mg) was further subjected to chromatographic principle, eluting with CH_2Cl_2–EtOAc, followed by Sephadex LH20 CC to give nummularine H (**3**, 9 mg). Subfraction 5 (509 mg) was further separated with silica gel CC using CH_2Cl_2–EtOAc mixtures of increasing polarity to give thirteen fractions (frs 5a to 5 m). Compound **1** (14.1 mg) precipitated out from fraction 5f (35 mg). Compound **5** (40.7 mg) was obtained from repeated Sephadex LH20 CC of fraction 5j (80 mg). A portion of subfraction 6 (338 mg) was further separated by CC eluting with EtOAc–MeOH to afford **2** (51.7 mg). The major metabolite **4** (117.5 mg) was furnished from two successive silica gel CC of fraction 3 (3.75 g) using EtOAc–MeOH as a solvent. Small quantities of the cyclopeptide alkaloids **1–5** and other type of compounds were also yielded in other fractions. It was worth noting that the alkaloids **2** and **5** which possessed a tryptophan unit gave a characteristic orange coloration with anisaldehyde–H_2SO_4 reagent, where as compounds **1**, **3** and **4** showed blue coloration with the same reagent.

4.3.1. Mauritine L (**1**)

Colorless solid; mp 236–237 °C; $[\alpha]_D^{27}$ = −56.3 (*c* 0.31, MeOH); UV (MeOH) λ_{max} (log ε) end absorption; CD (MeOH) $\Delta\varepsilon$ 279 (−18.26), 239 (−64.13) nm; IR (KBr) ν_{max} 3291, 2964, 2937, 2878, 1679, 1633, 1535, 1508, 1237 and 695 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; ESMS *m/z* 521 $[M+H]^+$; HRTOFMS (APCI$^+$) *m/z* 521.3124 $[M+H]^+$ (calcd. for $C_{30}H_{40}N_4O_4$ + H, 521.3122).

4.3.2. Mauritine M (**2**)

Colorless solid, mp 188–189 °C; $[\alpha]_D^{27}$ = −385.5 (c 0.28, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.7), 272 (4.2), 289 (4.0), 318 (3.9) nm; CD (MeOH) $\Delta\varepsilon$ 320 (−7.30), 263 (−16.83), 228 (+8.10), 209 (−10.73) nm; IR (KBr) ν_{max} 3345, 2963, 1679, 1664, 1637, 1508, 1458, 1438, 1224, 1186, 1038 and 741 cm^{-1}; EIMS *m/z* 686 $[M]^+$(1), 587 (7), 549 (100), 395 (20), 324 (71), 248 (76), 216 (18), 170 (90), 130 (87), 100 (47); for ^1H and ^{13}C NMR spectroscopic data, see Table 1; ESMS *m/z* 687 $[M+H]^+$; HRTOFMS (APCI$^+$) *m/z* 687.3856 $[M+H]^+$ (calcd. for $C_{38}H_{50}N_6O_6$ + H, 687.3856).

4.3.3. Additional data for nummularine B (**4**)

Colorless solid, mp 219–220 °C; $[\alpha]_D^{27}$ = −507.0 (c 0.29, MeOH); UV (MeOH) λ_{max} (log ε) 268 (4.1), 319 (3.9) nm; CD (MeOH) $\Delta\varepsilon$ 319 (−13.00), 264 (−27.58), 231 (+2.19), 217 (−21.81) nm; IR (KBr) ν_{max} 3409, 3325, 2966, 1693, 1651, 1642, 1528, 1514, 1421, 1369, 1227, 1182, 1041, 773 and 700 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; ESMS *m/z* 592 $[M+H]^+$; HRTOFMS (APCI$^+$) *m/z* 592.3122 $[M+H]^+$ (calcd. for $C_{32}H_{42}N_5O_6$ + H, 592.3130).

4.3.4. Methylation of **4**: nummularine B methiodide (**4′**)

A mixture of compound **4** (40 mg) and excess MeI (1 mL) was stirred at room temperature for 48 h. The reaction mixture was evaporated to dryness and subsequently purified by alumina CC (eluting with a gradient system of EtOAc–MeOH) and recrystallized by dissolving **4′** in MeOH–CH_2Cl_2, followed by addition of H_2O until turbidity was observed to obtain the *N,N*-dimethylated analogue, nummularine B methiodide (**4′**, 20 mg; 48%) as colorless needles, mp 190–192 °C, $[\alpha]_D^{27}$ = −337.0 (c 0.32, MeOH); UV (MeOH) λ_{max} (log ε) 269 (3.9), 319 (3.8) nm; IR (KBr) ν_{max} 3468, 3393, 3276, 2966, 1676, 1638, 1510, 1219, 1104 and 1025 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; ESMS *m/z* 620 $[M]^+$ (100); HRTOFMS (APCI$^+$) *m/z* 620.3428 (calcd. for $C_{34}H_{46}N_5O_6$, 620.3443).

4.3.5. Crystal data of nummularine B methiodide (**4′**)

$C_{34}H_{46}N_5O_6{\cdot}I$, M_r = 747.67, orthorhombic, space group $P2_12_12_1$ (No. 19) with *a* = 11.0721 (2) Å, *b* = 17.4815 (5) Å, *c* = 19.5641 (6) Å, *V* = 3786.8 (2) Å^3, *Z* = 4, D_{calc} = 1.311 Mg/m^3. F_{000} = 1544, μ = 0.892 mm^{-1}. Data collection and reduction: crystal size 0.10 × 0.15 × 0.20 mm, θ range 1.00–26.44°, 28,596 reflection collected, 4317 independent reflections (R_{int} = 0.048), final *R* indices ($I > 2\sigma(I)$: 0.0603, wR_2 = 0.1939 for 416 parameters, GOF = 1.011. Flack parameter = −0.01 (3). Intensity data were measured on a Bruker-Nonius kappaCCD diffractometer. Crystallographic data for the structure **4′** in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC-779370. Copies of the data can be obtained, free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

4.4. Bioassay procedure

Antiplasmodial activity was evaluated against the parasite *P. falciparum* (K1, multidrug resistant strain). *P. falciparum* was maintained continuously according to the method of Trager and Jensen (1976). Quantitative assessment of antiplasmodial activity *in vitro* was determined by means of the microculture radioisotope technique according to Desjardins et al. (1979). The concentration causing 50% inhibition of parasite growth was indicated by the *in vitro* uptake of 3[H]-hypoxanthine by *P. falciparum*.

Dihydroartemisinin, the standard compound for antiplasmodial test, exhibited an IC_{50} value of 4.2 nM. The antimycobacterial activity was assessed against *M. tuberculosis* $H_{37}Ra$ using the Microplate Alamar Blue Assay (Collins and Franzblau, 1997). Isoniazid and kanamycin sulfate, the standard drugs for the antimycobacterial assay, showed the respective MIC of 0.4 and 4.2 μM.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2011.03.003.

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