14-Membered Cyclopeptides from *Paliurus ramosissimus* and *P. hemsleyanus*

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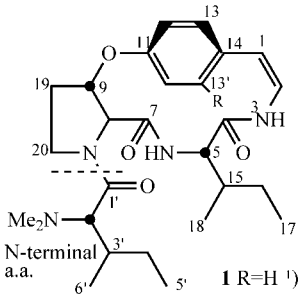
Six 14-membered cyclopeptide alkaloids, *i.e.*, ramosines A–C, mucronine J, and lotusines A and D, were isolated from the roots of *Paliurus ramosissimus*, and an additional four, hemsines A–D, from the roots of *P. hemsleyanus*. Among these, ramosines A–C (**1**, **5**, and **6**, resp.) and hemsines A and B (**7** and **8**, resp.) are new bases of the amphibine-B type, and hemsines C and D (**9** and **10**, resp.) are new integerrine-type alkaloids. Additionally, ramosine C (**6**) represents the first 14-membered cyclopeptide alkaloid possessing a substitution (–OH) at C(13′). Their structural elucidations were based on spectral analysis and molecular-modeling studies. Pronounced solvent effects in the ^{1}H- and ^{13}C-NMR spectra of these two types of alkaloids were observed.

1. Introduction. – Our previous studies on the chemical constituents of *Paliurus ramosissimus* (LOUR.) POIR. (Rhamnaceae) have resulted in the isolation from its root and stem of triterpenes of the ceanothane and lupane types [1][2], and of 13-membered cyclopeptide alkaloids of the zizyphine type [3][4]. Some of these alkaloids showed either shortening or prolonging of the methahexital-induced sleeping time in mouse experiments [4]. During the study of 13-membered bases from the root, we found that several alkaloids of a different type were also present. Further separation of these fractions yielded six 14-membered alkaloids **1**–**6**. To enrich the chemical bank of these cyclopeptides, we also studied the chemical constituents of a related plant, *P. hemsleyanus* REHD., a deciduous tree widely distributed in southern China [5]. This work resulted in the isolation of four 14-membered cyclopeptides **7**–**10**, and an aporphine nuciferine [6], in addition to the reported ceanothic acid esters [7]. In the present paper, we report the structure characterization of these compounds.

2. Results and Discussion. – Compounds **1**–**8** belong to 14-membered cyclopeptide alkaloids of the amphibine-B type, according to the common spectral properties [8][9] summarized in the following. The IR spectra of **1**–**8** showed absorptions at *ca.* 3300–3310, 1680–1690, and 1630–1655 cm^{-1} for the amide (CONH) function, and 1220–1230 cm^{-1} for the aryl alkyl ether function. The UV spectra revealed absorption maxima at *ca.* 212 and 240 nm, reflecting the chromophore of a 4-oxystyrylamine moiety in conjugation with the peptide chain, and the CD spectra exhibited negative and positive *Cotton* effects (CE) at *ca.* 235 and 276 nm, respectively, consistent with the same (5*S*,8*S*,9*S*)-configuration[1]) present in the 14-membered ring moiety of amphib-

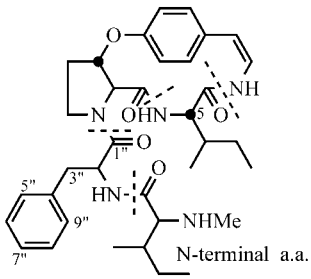
[1]) Arbitrary numbering.

ine-B-type alkaloids. Among these eight cyclopeptides, compounds **2**–**4** were identified as mucronine J [10] and lotusines D and A [11], respectively, by comparing their physical data with those reported.



1 R=H [1])

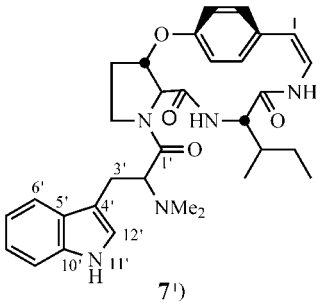
	R	N-terminal a.a.
2	H	Me_2N-L-Leu
3	H	MeNH-L-Phe
4	H	Me_2N-L-Phe
6	OH	Me_2N-L-Phe



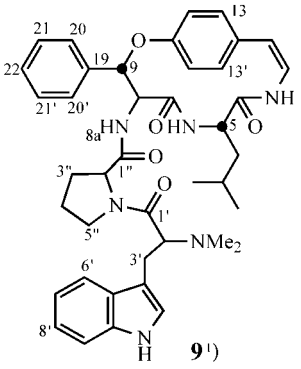
5 [1])

The ^{1}H-NMR spectra of **1**–**8**[1]) showed common signals for an *ABX* system consisting of H–C(1) (*δ ca.* 6.32, *d*), H–C(2) (*δ ca.* 6.71, *dd*), and H–C(3) (*δ ca.* 6.32, *d*), and an aromatic *ABCD* system consisting of H–C(12) (*δ ca.* 7.28, *dd*), H–C(12′) (*δ ca.* 7.12, *dd*), H–C(13) (*δ ca.* 7.08), and H–C(13′) (*ca.* *δ* 7.12, br. *d*), the latter system being replaced by an *ABX* spin system in **6**. The coupling of H–C(8) (*δ ca.* 4.38, *d*, *J* = 5.2 Hz) with H–C(9) (*δ ca.* 5.41, *ddd*, *J* = 13.6, 6.9, 5.2 Hz) of the (3*S*)-3-hydroxyproline unit showed different coupling constants from those of the corresponding H–C(9) signal (*δ* 5.40, *ddd*, *J* = 7.2, 7.2, 3.0 Hz) in paliurines A–F, the 13-membered zizyphine-A-type cyclopeptides isolated from the same plant [3]. Finally, two Me signals, 1 *d* (*δ ca.* 0.75) and 1 *t* (*δ ca.* 0.85), were observed for the exocyclic isoleucine unit. The coupling relationships of these protons were verified by COSY-45 spectra.

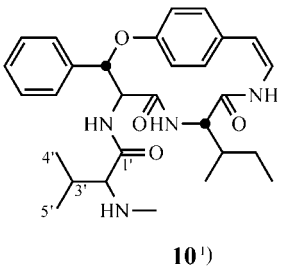
Compound **1** had a molecular formula $C_{27}H_{40}N_4O_4$, as deduced from its HR-EI-MS. Besides the skeletal signals mentioned above, the ^{1}H-NMR spectrum of **1** ($CDCl_3$) (*Table 1*) revealed additional signals for two Me groups (*δ* 0.66 (*d*, *J* = 6.5 Hz) and 0.83 (*t*, *J* = 7.1 Hz)) and a Me_2N group (*δ* 2.34 (*s*, 6 H)). These data suggested an *N*,*N*-dimethylisoleucine unit for the N-terminal amino acid. The FAB-MS of **1** revealed a base peak at *m*/*z* 114, corresponding to the fragment ion **a** from this residue. Hence, compound **1** had the proposed structure, leaving the C(2′) configuration undetermined.



7 [1])



9 [1])



10 [1])

Table 1. *^{1}H- and ^{13}C-NMR Data for the N-Terminal Amino Acids in* **1**[1]) *and* **5**[a])[1]) *in $CDCl_3$.* δ in ppm, J in Hz.

	1 (Me_2N-Ile)		**5**[a]) (MeNH−Ile)	
	^{1}H	^{13}C[b])	^{1}H	^{13}C[b])
C(1′)		171.9 (*s*)		173.4 (*s*)
H−C(2′)	3.06 (*d*, *J* = 9.8)	68.1 (*d*)	2.79 (*d*, *J* = 4.6)	69.8 (*d*)
H−C(3′)	1.80 (*m*)	34.6 (*d*)	1.63 (*m*)	38.1 (*d*)
CH_2(4′)	1.10, 1.63 (2*m*)	25.5 (*t*)	0.95, 1.18 (2*m*)	24.9 (*t*)
Me(5′)	0.83 (*t*, *J* = 7.1)	10.5 (*q*)	0.78 (*t*, *J* = 0.76)	11.7 (*q*)
Me(6′)	0.66 (*d*, *J* = 6.5)	15.5 (*q*)	0.75 (*d*, *J* = 7.2)	15.6 (*q*)
MeN	2.34 (*s*)	41.6 (*q*)	2.37 (*s*)	36.1 (*q*)

[a]) Data of the intermediate amino acid, Phe, in **5**. ^{1}H-NMR: 4.95 (*dd*, *J* = 15.4, 7.7, H−C(2″)); 7.68 (*d*, *J* = 8.4, NH−C(2″)); 2.82 (*d*, *J* = 7.7, H−C(3″)); 7.06 (br. *d*, *J* = 7.4, H−C(5″), H−C(9″)); 7.21 (*m*, H−C(6″), H−C(7″), H−C(8″). ^{13}C-NMR: 171.1 (*s*, C(1″)); 50.9 (*d*, C(2″)); 38.7 (*t*, C(3″)); 135.9 (*s*, C(4″)); 129.1 (*d*, C(5″)); C(9″)); 128.6 (*d*, C(6″), C(8″)); 127.1 (*d*, C(7″)). [b]) Multiplicity was obtained from DEPT experiments.

In addition, a molecular-modeling study coupled with consideration of its CD spectra indicated an α-helical arrangement for the peptide chain, which requires that the terminal basic amino acid must be in the L-form, *i.e.* Me_2N-L-Ile with (2′*S*)-configuration.

The ^{1}H- and ^{13}C-NMR assignment of **1** (*Tables 1* and *2*) were accomplished by analysis of the COSY-45, NOESY, HMQC, and HMBC spectra. Thus, the recognition of the chemical shifts of H−C(12) (δ 7.28) and H−C(12′) (δ 7.13) were achieved by their NOE relationship to H−C(9) (δ 5.54) and H−C(8) (δ 4.35), respectively, which in turn established the chemical shifts of C(12) (δ 122.8) and C(12′) (δ 123.0) from inspection of the HMQC spectrum. This observation was also supported by the NOE relationships H_α−C(20)/Me_2N−C(2′) and H−C(2′), H_α−C(20)/H_α−C(19), and H_α−C(19)/H−C(8), thus confirming the structure of **1**.

Compound **5** had a molecular formula $C_{35}H_{47}N_5O_5$, based on its HR-FAB-MS. Besides the signals indicated above, its ^{1}H-NMR spectrum (*Table 1*) displayed characteristic signals for the N-terminal dipeptide moiety. The MS confirmed a terminal MeNH-Ile moiety with a base peak at *m*/*z* 100 for the fragment ion **b**. A molecular model of this compound also suggested an α-helix for its peptide chain as revealed by a positive CE at 220 nm and a negative CE at 237 nm in its CD spectrum. Consequently, the structure of **5** was determined as shown.

The five aromatic protons (δ 7.06 (2 H), 7.21 (3 H)), two Me groups (δ 0.78 (*t*), 0.75 (*d*)), one *Me*NH (δ 2.37), and H−C(α) (δ 2.79 (*d*)) of the N-terminal dipeptide suggested a MeNH−Ile−Phe moiety for **5**.

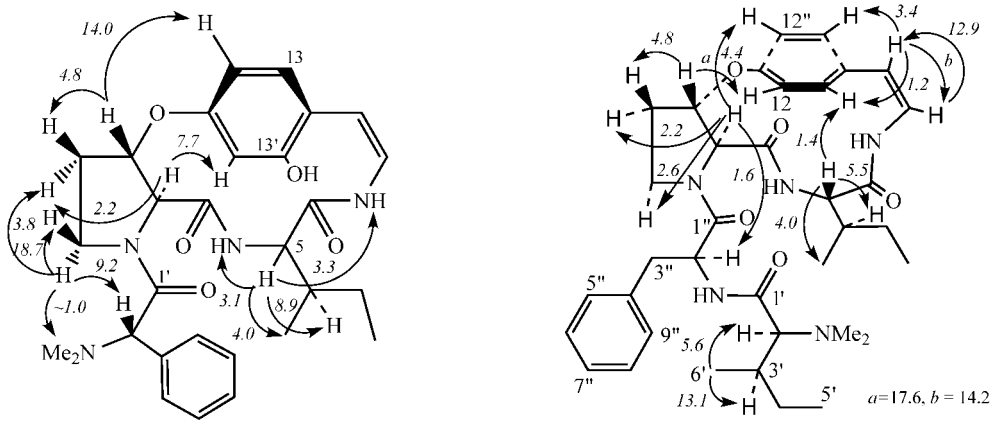
Compound **6** had a molecular formula $C_{30}H_{38}N_4O_5$, based on its HR-FAB-MS. Its FAB-MS exhibited a base peak at *m*/*z* 148, compatible with a fragment ion **c** that established an *N*,*N*-dimethylphenylalanine unit as the N-terminal amino acid residue. It contained a phenolic moiety as the UV spectrum showed a bathochromic shift under strong alkaline conditions. The α-helical model of **6** was reflected in the CD spectrum which showed a positive CE at 226 nm and a negative CE at 247 nm, indicating (2′*S*)-configuration for the terminal basic amino acid, *i.e.*, Me_2N-L-Phe. The remaining data confirmed the structure of **6**.

Table 2. *^{1}H- and ^{13}C-NMR Data and HMBC of* **6**[1]) *in $CDCl_3$.* δ in ppm, J in Hz.

	^{1}H[a])	^{13}C[b])	HMBC (H → C)		^{1}H[a])	^{13}C[b])	HMBC (H → C)
H–C(1)	6.00 (*d*, *J* = 7.5)	107.2 (*d*)	C(2), C(13′), C(14)	C(14)		117.5 (*s*)	
H–C(2)	6.90 (*dd*, *J* = 10.4, 7.5)	128.3 (*d*)	C(1)	H–C(15)	2.09 (*m*)	35.6 (*d*)	
H–N(3)	6.74 (*d*, *J* = 10.4)			CH_2(16)	1.28 (*m*, H_α), 1.14 (*m*, H_β)	24.2 (*t*)	C(17)
C(4)		167.5 (*s*)		Me(17)	0.86 (*t*, *J* = 7.4)	12.2 (*q*)	C(15), C(16)
H–C(5)	4.16 (*dd*, *J* = 8.8, 2.6)	58.6 (*d*)	C(4), C(15), C(17)	Me(18)	0.62 (*d*, *J* = 7.0)	16.0 (*q*)	C(5), C(15), C(16)
H–N(6)	6.69 (*d*, *J* = 8.8)			CH_2(19)	2.03 (*m*, H_α), 2.45 (*ddd*, *J* = 12.0, 6.9, 5.4, H_β)	31.9 (*t*)	
C(7)		171.0 (*s*)		CH_2(20)	4.17 (*dd*, *J* = 12.7, 8.6, H_α), 3.01 (*ddd*, *J* = 12.7, 11.2, 5.1 H_β)	45.8 (*t*)	C(8), C(9)
H–C(8)	4.31 (*d*, *J* = 5.2)	64.0 (*d*)	C(7), C(9), C(20), C(21)	C(1′)		171.0 (*s*)	
H–C(9)	5.41 (*ddd*, *J* = 13.6, 6.9, 5.2)	84.4 (*d*)	C(7)	H–C(2′)	3.42 (*dd*, *J* = 9.2, 3.7)	67.4 (*d*)	C(1′), *Me*$_2$N–C(2′)
C(11)		159.5 (*s*)		CH_2(3′)	3.02 (*dd*, *J* = 13.6, 9.2, H_α), 2.89 (*dd*, *J* = 13.6, 3.7, H_β)	30.3 (*t*)	C(1′), C(2′), C(4′), C(5′), C(9′)
H–C(12)	6.85 (*dd*, *J* = 8.4, 2.2)	115.2 (*d*)	C(11), C(12′), C(13), C(14)	C(4′)		138.8 (*s*)	
H–C(12′)	6.73 (*d*, *J* = 2.2)	110.7 (*d*)	C(11), C(12), C(14)	H–C(5′), H–C(9′)	7.06 (br. *d*, *J* = 7.6)	129.0 (*d*)	C(3′), C(6′), C(7′), C(8′)
H–C(13)	6.91 (*d*, *J* = 8.4)	132.9 (*d*)	C(11), C(13′), C(14)	H–C(6′), H–C(8′)	7.19 (*t*, *J* = 7.6)	128.5 (*d*)	C(4′), C(5′), C(7′), C(9′)
H–C(13′)		154.6 (*s*)		H–C(7′)	7.12 (*t*, *J* = 7.6)	126.3 (*d*)	C(5′), C(6′), C(8′), C(9′)
				Me_2N	2.38 (*s*)	41.5 (*q*)	C(2′)

[a]) Typical ^{1}H-NMR data of the common ring moiety at **1**–**5**, for example in **1**, are as follows: 6.26 (*d*, *J* = 7.8, H–C(1)); 6.72 (*dd*, *J* = 10.2, 7.8, H–C(2)); 6.49 (*d*, *J* = 10.2, H–C(3)); 7.28 (*dd*, *J* = 8.4, 2.2, H–C(12)); 7.13 (*m*, H–C(12′)); 7.08 (br. *d*, *J* = 8.4, H–C(13)); 7.12 (*m*, H–C(13′)). [b]) Multiplicity was obtained from DEPT experiments. Typical ^{13}C-NMR data of the common ring moiety in **1**–**5**, for example in **1**, are as follows: 114.1 (*d*, C(1)); 125.7 (*d*, C(2)); 157.6 (*s*, C(11)); 122.8 (*d*, C(12)); 123.0 (*d*, C(12′)); 132.6 (*d*, C(13)); 130.3 (*d*, C(13′)); 132.8 (*s*, C(14)).

The ^{1}H-NMR spectrum of **6** showed an aromatic *ABX* system at δ 6.73 (*d*, *J* = 2.2 Hz, H–C(12′)), 6.85 (*dd*, *J* = 8.4, 2.2 Hz, H–C(12)), and 6.91 (*d*, *J* = 8.4 Hz, H–C(13)) instead of the *ABCD* system in the previous series of compounds (*Table 2*). The spin system was accommodated by a 1,2,4-trisubstituted styrylamine moiety. Since the coupling patterns of the H–C(8) and H–C(9) signals indicated a 14-membered cyclopeptide for **6**, either position C(13) or C(13′) could be OH-substituted. This position was determined by NOE experiments



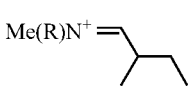
6[1]) **8**[1])

Fig. 1. *Key NOEs* (%, italics) *and conformations of* **6** *in* $CDCl_3$ *and of* **8** *in* CD_3OD

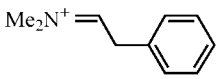
(*Fig. 1*). Irradiation of H–C(9) (δ 5.41) enhanced the H–C(12) signal (δ 6.85, *dd*; 14.0%), while irradiation of H–C(8) (δ 4.31) enhanced the signal of *meta*-coupled H–C(12′) (δ 6.73, *d*; 7.7%), indicating C(13′) to be OH-substituted. The HMBC spectrum revealed ^3J correlations of H–C(13) (δ 6.91) to C(11) (δ 159.5, *s*) and C(13′) (δ 154.6), and H–C(1) (δ 6.00, *d*) to C(13′) (*Table 2*), also confirming this structural feature. The (2′*S*)-configuration was also supported by an NOE experiment in which the signals of H–C(2′) (δ 3.42, *dd*) and *Me*$_2$N–C(2′) (δ 2.38, *s*) were enhanced upon irradiation at the frequency of H_α–C(20) (δ 4.17).

Compound **7** had a molecular formula $C_{32}H_{40}N_5O_4$, based on its HR-FAB-MS. Its FAB-MS exhibited a base peak at *m*/*z* 187, consistent with a fragment ion **d** and with an *N*,*N*-dimethyltryptophan as the N-terminal amino acid residue. The presence of this terminal moiety was also confirmed by the ^{1}H-NMR spectrum (D_2O-exchangeable indole NH at δ 8.08 (br. *s*)) and the adjacent H–C(12′) at δ 6.73 (*d*, $J = 1.8$ Hz) [12]). The CD spectrum of **7** revealed a positive CE at 223 nm and a negative CE at 236 nm, reflecting characteristics of the α-helix peptide chain and a Me_2N-L-Trp constituting the terminal amino acid residue. Hence the structure of **7** was established. The complete ^{1}H- and ^{13}C-NMR data were assigned by analysis of the 2D NMR spectra (COSY, TOCSY, HMQC, and HMBC), and the results are listed in *Table 3*.

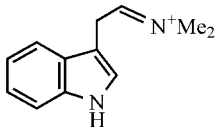
Compound **8** had a molecular formula $C_{36}H_{49}N_5O_5$, based on its HR-FAB-MS. Its ^{1}H-NMR spectrum (*Table 3*) exhibited additional signals typical for either a Me_2N–Phe–Ile or Me_2N–Ile–Phe as the terminal dipeptide moiety, besides the signals mentioned above for the 14-membered skeleton. Since the FAB-MS of **8** exhibited a base peak at *m*/*z* 114 consistent with a fragment ion **a**, the N-terminal dipeptide was established to be Me_2N–Ile–Phe. Hence, the structure of **8** was established as shown. The complete ^{1}H- and ^{13}C-NMR data of **8** were assigned by analysis of the 2D NMR spectra (COSY, TOCSY, HMQC, and HMBC) as listed in *Table 3*. To clarify some assignments, such as those of H–C(12), H–C(12′), H–C(13), and H–C(13′) and study the conformation, some selective NOE experiments in CD_3OD were undertaken (see *Fig. 1*). The configuration for the terminal dipeptide was established by comparison of its ^{13}C-NMR data with those of independently prepared



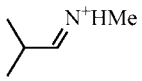
a R= Me, *m/z* 114
b R= H, *m/z* 100



c, *m/z* 148



d, *m/z* 187



e, *m/z* 86

dipeptide Me_2N-L$-$Ile$-$L$-$Phe$-$OEt [3]. It has been demonstrated that the C(2) chemical shift of the N-terminal amino acid in the dipeptide will be influenced to a greater extent due to the chirality difference of the composed amino acid residue. Thus, the identical chemical shift of C(2′) of **8** (δ 74.4 in $CDCl_3$) as compared to the corresponding signal in paliurine A, isolated from *P. ramossisimus* [3], supported the presence of the N-terminal dipeptide Me_2N-L$-$Ile$-$L$-$Phe. The CD spectrum of **8** showed a positive CE at 220 nm and a negative CE at 238 nm, conforming to an α-helical model for the peptide chain with L-configurations in the terminal dipeptide which is also corroborated by molecular modeling[2]).

A striking ^{1}H-NMR difference between the data of **8** measured in $CDCl_3$ and CD_3OD was observed. Particularly, in the more polar CD_3OD, the signals of H$-$C(1) and H$-$C(2) were shifted downfield (δ 6.67 *vs.* 6.29) and upfield (δ 6.28 *vs.* 6.73), respectively, relative to the corresponding signals in $CDCl_3$. This phenomenon was also found in ^{13}C-NMR spectra, in which a large downfield shift for the C(1) signal (δ 127.7 *vs.* 114.4) was observed. Other signals significantly shifted due to this solvent effect are those of C(4), C(7), C(8), C(12), C(15), and C(4″) (*Table 3*). Such a solvent effect was also observed for compound **10** in (D_5)pyridine (see below).

Compounds **9** and **10**[1]) belong to 14-membered cyclopeptides of the integerrine type, as exemplified by the typical spectral properties [8][9].

The IR spectra of **9** and **10** displayed absorptions at 3300–3310, 1680–1690, and 1630–1655 cm^{-1} for the amide (CONH) function, at 1220–1230 cm^{-1} for the aryl alkyl ether function, and at 750 and 710 cm^{-1} for the monosubstituted phenyl group. The UV spectra exhibited an absorption maximum at 224 nm and the CD spectra a weak negative CE at 285 nm and a strong negative CE at 237 nm, indicating the same (5*S*,8*S*,9*S*)-configuration of the 14-membered cyclopeptide moiety. The ^{1}H-NMR spectra revealed an *ABX* system for H$-$C(1), H$-$C(2) and H$-$N(3), an *ABCD* system for H$-$C(12), H$-$C(12′), H$-$C(13), and H$-$C(13′), an *AMX* system for H$-$C(8), H$-$C(9), and H$-$N(8) (H$-$N(8a)), and five aromatic protons for the Ph group of the (βS)-β-hydroxyphenylalanine unit. The relationships of these coupling systems were verified by a COSY-45 spectrum.

Compound **9** had a molecular formula $C_{41}H_{48}N_6O_5$, as deduced from its HR-FAB-MS. Beside the general signals indicated above for the integerrine-type skeleton, its ^{1}H-NMR spectrum ($CDCl_3$) revealed characteristic signals (*Table 4*) for an *N*,*N*-dimethyltryptophan (Me_2N-Trp) residue similar to those in compound **7** (*Table 3*) and a ring-incorporated leucine residue. All the data were consistent with the proposed structure **9**.

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[2]) Molecular modeling was performed by the simulated annealing program of Sybyl with the *Tripos* force fields (*Tripos, Inc.*), and the energy-minimized conformation was obtained after adjusting for the NOE data.

Table 3. *^{1}H- and ^{13}C-NMR Data for Compounds* **7**[a])[1]) ($CDCl_3$) *and* **8**[1]) ($CDCl_3$ and CD_3OD)

	8				
	^{1}H		^{13}C[b])		
	$CDCl_3$	CD_3OD	$CDCl_3$	CD_3OD	$\Delta\delta$
H−C(1)	6.29 (*d*, *J* = 7.8)	6.67 (*d*, *J* = 7.5)	114.4 (*d*)	127.7 (*d*)	− 13.3
H−C(2)	6.73 (*dd*, *J* = 10.2, 7.8)	6.28 (*d*, *J* = 7.5)	125.5 (*d*)	126.4 (*d*)	− 0.9
H−N(3)	6.49 (*d*, *J* = 10.2)				
C(4)			166.9 (*s*)	171.3 (*s*)	− 4.4
H−C(5)	4.13 (*dd*, *J* = 10.4, 8.7)	3.98 (*d*, *J* = 6.9)	59.0 (*d*)	59.6 (*d*)	− 0.6
H−N(6)	6.55 (*d*, *J* = 8.7)				
C(7)			170.3 (*s*)	173.1 (*s*)	− 2.8
H−C(8)	4.24 (*d*, *J* = 5.6)	4.13 (*d*, *J* = 6.3)	63.9 (*d*)	66.5 (*d*)	− 2.6
H−C(9)	5.50 (*ddd*, *J* = 9.3, 7.1, 6.4)	5.34 (*dt*, *J* = 10.3, 6.3)	83.4 (*d*)	84.0 (*d*)	− 0.6
C(11)			157.4 (*s*)	158.3 (*s*)	− 0.9
H−C(12)	7.18 (*m*)	7.17 (br. *d*, *J* = 8.4)	122.7 (*d*)	120.1 (*d*)	+ 2.6
H−C(12′)	7.18 (*m*)	7.045 (*dd*, *J* = 8.1, 2.4)	122.8 (*d*)	122.5 (*d*)	+ 0.3
H−C(13)	7.07 (*m*)	7.051 (*dd*, *J* = 8.0, 1.5)	132.5 (*d*)	132.2 (*d*)	+ 0.3
H−C(13′)	7.07 (*m*)	7.10 (*dd*, *J* = 8.0, 1.9)	130.2 (*d*)	130.9 (*d*)	− 0.7
C(14)			132.6 (*s*)	133.4 (*s*)	− 0.8
H−C(15)	2.14 (*m*)	1.74 (*m*)	35.3 (*d*)	38.4 (*d*)	− 3.1
CH_2(16)	1.25, 1.08 (2*m*)	1.44, 1.13 (2*m*)	23.8 (*t*)	25.3 (*t*)	− 1.5
Me(17)	0.87 (*t*, *J* = 7.3)	0.83 (*t*, *J* = 7.6)	12.2 (*q*)	11.6 (*q*)	+ 0.6
Me(18)	0.80 (*d*, *J* = 6.9)	0.82 (*d*, *J* = 7.1)	16.0 (*q*)	15.7 (*q*)	+ 0.3
CH_2(19)	2.47 (*ddd*, *J* = 12.0, 6.6, 5.4, H_β), 2.20 (*m*, H_α)	2.18 (*m*, H_α), 2.53 (*dt*, *J* = 11.6, 5.7, H_β)	31.9 (*t*)	32.7 (*t*)	− 0.8
CH_2(20)	4.19 (*dd*, *J* = 8.5, 2.8, H_α), 2.88 (*ddd*, *J* = 13.1, 11.3, 5.3, H_β)	4.25 (*dd*, *J* = 9.1, 9.8, H_α), 3.42 (*ddd*, *J* = 12.2, 10.8, 5.2, H_β)	46.4 (*t*)	46.9 (*t*)	− 0.5
C(1″)			171.1 (*s*)	171.9 (*s*)	− 0.8
H−C(2″)	4.94 (*dd*, *J* = 8.1, 7.6)	4.90 (*dd*, *J* = 9.4, 5.2)	51.0 (*d*)	52.9 (*d*)	− 1.9
CH_2(3″)	2.83 (*m*)	2.98 (*dd*, *J* = 14.0, 5.2), 2.75 (*dd*, *J* = 14.0, 9.4)	38.7 (*t*)	38.3 (*t*)	+ 0.4
C(4″)			135.8 (*s*)	138.2 (*s*)	− 2.4
H−C(5″), H−C(8″))	7.20 (*m*)	7.22 (*m*)	128.7 (*d*)	130.3 (*d*)	− 1.6
H−C(6″), H−C(9″))	7.20 (*m*)	7.23 (*m*)	129.1 (*d*)	129.5 (*d*)	− 0.4
H−C(7″)	7.20 (*m*)	7.18 (*m*)	127.2 (*d*)	127.8 (*d*)	− 0.6
C(1′)			171.8 (*s*)	173.2 (*s*)	− 1.4
H−C(2′)	2.51 (*d*, *J* = 5.3)	2.64 (*d*, *J* = 8.8)	74.4 (*d*)	73.9 (*d*)	+ 0.5
H−C(3′)	1.69 (*m*)	1.76 (*m*)	34.3 (*d*)	34.7 (*d*)	− 0.4
CH_2(4′)	0.95, 1.25 (2*m*)	1.55, 1.05 (2*m*)	26.9 (*t*)	26.5 (*t*)	+ 0.4
Me(5′)	0.85 (*t*, *J* = 7.4)	0.87 (*t*, *J* = 7.5)	12.0 (*q*)	11.2 (*q*)	+ 0.8
Me(6′)	0.58 (*d*, *J* = 6.8)	0.75 (*d*, *J* = 6.7)	14.0 (*q*)	15.5 (*q*)	− 1.5
Me_2N	2.19 (*s*)	2.03 (*s*)	43.2 (*q*)	42.2 (*q*)	+ 1.0

[a]) ^{1}H-NMR of **7**: δ of H−C(1) to CH_2(16) similar to those of **8** ($CDCl_3$): 0.78 (*t*, *J* = 7.4, Me(17)); 0.57 (*d*, *J* = 7.0, Me(18)); 2.36 (*dt*, *J* = 9.4, 5.6, H_β−C(19)); 2.03 (*m*, H_α−C(19)); 3.95 (*dd*, *J* = 10.5, 8.2, H_α−C(20)); 2.73 (*ddd*, *J* = 12.1, 11.6, 5.4, H_β−C(20)); 3.63 (*dd*, *J* = 7.5, 5.6, H−C(2′)); 3.11 (*m*, H−C(3′)); 7.54 (br. *d*, *J* = 7.8, H−C(6′)); 7.09 (*m*, H−C(7′)); 7.17 (*m*, H−C(8′)); 7.29 (br. *d*, *J* = 7.2, H−C(9′)); 8.08 (br. *s*, H−N(11′)); 6.73 (*d*, *J* = 1.6, H−C(12′)); 2.46 (*s*, Me_2N−C(2′)). ^{13}C-NMR of **7**: δ of C(1) to C(20) similar to those of **8** ($CDCl_3$): 171.7 (*s*, C(1′)); 65.6 (*d*, C(2′)); 21.2 (*t*, C(3′)); 111.8 (*s*, C(4′)); 127.3 (*s*, C(5′)); 118.1 (*d*, C(6′)); 119.4 (*d*, C(7′)); 122.4 (*d*, C(8′)); 111.3 (*d*, C(9′)); 136.1 (*s*, C(10′)); 122.1 (*d*, C(12′)); 41.8 (*q*, *Me*$_2$N−C(2′)). [b]) Data were assigned from HMQC (^1J(C,H) and HMBC (^2J(C,H) and ^3J(C,H) (see *Exper. Part*).

Table 4. *[1]H-NMR and [13]C-NMR Data for Compounds* **9**[1]) *and* **10**[1]). δ in ppm, J in Hz.

	10 (C_5D_5N)		**9** ($CDCl_3$)			
	^1H	^{13}C	^1H[a])	^{13}C[b])	Key HMBC[b]) (H → C)	Key NOESY[b])
H–C(1)	6.84 (*d*, *J* = 7.3)	129.4 (*d*)	6.39 (*d*, *J* = 7.6)	114.9 (*d*)	C(2), C(14)	H–C(2), H–C(13), H–C(13′)
H–C(2)	6.56 (*dd*, *J* = 7.3, 3.0)	127.6 (*d*)	6.77 (*dd*, *J* = 10.4, 7.6)	125.8 (*d*)	C(14)	H–C(1)
H–N(3)	9.24 (*d*, *J* = 3.0)		6.61 (*d*, *J* = 10.4)		C(1)	
C(4)		170.2 (*s*)		167.5 (*s*)		
H–C(5)	4.64 (*t*-like, *J* = 9.0)	58.5 (*d*)	4.25 (*ddd*, *J* = 11.3, 6.4, 3.0)	53.0 (*d*)	C(4)	H–N(6) (vw)[c]), H–C(15)
H–N(6)	9.29 (*d*, *J* = 9.0)		6.36 (*d*, *J* = 6.4)		C(7)	H–C(5) (vw)[c])
C(7)		172.0 (*s*)		171.1 (*s*)		
H–C(8)	5.67 (*dd*, *J* = 9.7, 8.7)	57.5 (*d*)	4.52 (*dd*, *J* = 8.6, 6.4)	56.0 (*d*)	C(9), C(7), C(1″)	H–C(12′), H–C(20), H–C(20′))
H–N(8a)	8.63 (*d*, *J* = 9.7)		5.23 (*d*, *J* = 8.6)		C(1″)	H–C(20)(H–C(20′)(w)[c]), C(9)(w)[c])
H–C(9)	6.43 (*d*, *J* = 8.7)	81.9 (*d*)	5.74 (*d*, *J* = 6.4)	81.3 (*d*)	C(7), C(8), C(19), C(20)	H–C(12), H–C(20)(H–C(20′)), H–N(8a)
C(11)		156.2 (*s*)		155.0 (*s*)		
H–C(12)	7.58 (*m*)	118.3 (*d*)	7.40 (*dd*, *J* = 8.4, 2.4)	123.7 (*d*)	C(11), C(12′), C(14)	H–C(9)
H–C(12′)	7.03 (*dd*, *J* = 8.2, 2.0)	121.4 (*d*)	7.28 (br. *d*, *J* = 8.1)	124.0 (*d*)	C(11), C(12), C(14)	
H–C(13)	7.18 (*m*)	131.0 (*d*)	7.15 (*dd*, *J* = 8.4, 1.5)	130.6 (*d*)	C(1), C(11), C(13′)	
H–C(13′)	7.30 (*m*)	130.3 (*d*)	7.20 (*dd*, *J* = 8.1, 1.5)	132.1 (*d*)	C(1), C(11), C(13)	
C(14)		132.2 (*s*)		133.1 (*s*)		
H–C(15) or CH_2(15))	2.00 (*m*)	36.9 (*d*)	1.86, 1.35 (2*m*)	39.4 (*t*)		
CH_2(16) or H–C(16)	1.58, 1.17 (2*m*)	25.0 (*t*)	1.51 (*m*)	24.5 (*d*)		
Me(17)	0.70 (*t*, *J* = 7.4)	11.0 (*q*)	0.94 (*d*, *J* = 6.5)	21.1 (*q*)	C(15), C(16), C(18)	
Me(18)	0.91 (*d*, *J* = 6.7)	15.8 (*q*)	0.92 (*d*, *J* = 6.6)	23.5 (*q*)	C(15), C(16), C(17)	
C(19)		139.3 (*s*)		136.9 (*s*)		
H–C(20) (H–C(20′))	7.72 (br. *d*, *J* = 7.3)	129.2 (*d*)	6.38 (br. *d*, *J* = 7.3)	126.8 (*d*)	C(9), C(22)	
H–C(21) (H–C(21′))	7.37 (*t*-like, *J* = 7.3)	128.58 (*d*)	6.79 (*dd*, *J* = 7.7, 7.3)	128.7 (*d*)	C(19), C(20), C(22)	
H–C(22)	7.30 (*m*)	128.64 (*d*)	7.08 (*t*, *J* = 7.7)	128.5 (*d*)	C(21), C(20)	
N-Terminal a.a.	MeNH–Val		Me_2N–Trp			
C(1′)		172.8 (*s*)		170.1 (*s*)		
H–C(2′)	2.73 (*d*, *J* = 5.2)	70.7 (*d*)	3.18 (*m*)	67.6 (*d*)	Me_2N	Me_2N, H_α–C(5″)
H–C(3′) or CH_2(3′)	1.80 (*m*)	31.9 (*d*)	3.21 (*m*, H_α), 2.93 (*m*, H_β)	16.4 (*t*)	C(1′), C(2′), C(4′)	H–C(6′) (to H_β–C(3′))
Me(4′) or C(4′)	0.76 (*d*, *J* = 6.8)	19.9 (*q*)		111.9 (*s*)		
Me(5′) or C(5′)	0.71 (*d*, *J* = 6.8)	18.1 (*q*)		127.6 (*s*)		
H–C(6′)			7.65 (*d*, *J* = 7.8)	117.7 (*d*)	C(4′), C(8′), C(10′)	H_b–C(3′), H–C(7′)
H–C(7′)			7.22 (*m*)	119.5 (*d*)	C(5′), C(9′)	H–C(6′)
H–C(8′)			7.29 (br. *dd*, *J* = 8.4, 8.0)	122.3 (*d*)	C(6′), C(10′)	
H–C(9′)			7.58 (br. *d*, *J* = 8.0)	111.8 (*d*)	C(5′), C(7′)	H–C(8′), H–N(11′)
C(10′)				136.1 (*s*)		
H–N(11′)			8.92 (br. *s*)			H–C(9′), H–C(12′)
H–C(12′)			6.68 (*d*, *J* = 2.2)	124.8 (*d*)	C(9′), C(10′), C(5′)	H–C(2′), H–N(11′)
*Me*NH or Me_2N	2.07 (*s*)	35.7 (*q*)	2.39 (*s*)	41.4 (*q*)	C(2′)	

[a]) ^{1}H-NMR for intermediate amino acid (proline): 4.36 (*dd*, *J* = 8.7, 2.8, H–C(2″)); 1.78 (*m*, H_α–C(3″)); 1.64 (*m*, H_β–C(3″)); 1.42 (*m*, H_α–C(4″)); 0.65 (*m*, H_β–C(4″)); 3.33 (*dd*, *J* = 16.9, 9.3, H_α–C(5″)); 2.01 (*dt*, *J* = 3.2, 9.3, H_β–C(5″)). [b]) Data for intermediate amino acid (proline): ^{13}C-NMR: 172.0 (*s*, C(1″)); 59.7 (*d*, C(2″)); 29.1 (*t*, C(3″)); 23.7 (*t*, C(4″)); 45.8 (*t*, C(5″). HMBC: H–C(2″) → C(1″), C(3″), and C(4″); H_α–C(3″) → C(1″), H_α–C(5″) → C(4″), H_β–C(5″) → C(3″). NOESY: H–C(2″) → H_α–C(3″), H_β–C(3″) → H_β–C(4″), H_α–C(4″) → H_α–C(5″). [c]) vw = very week, w = week.

In the ^{1}H-NMR spectrum of **9**[1]), H–C(5) of the Leu residue appeared at δ 4.25 (*ddd*), besides two Me *d* at δ 0.94 and 0.92. The MS confirmed the Me_2N–Trp moiety as the N-terminal amino acid residue by displaying a base peak at *m*/*z* 187, consistent with fragment ion **d**. The intermediate amino acid residue was deduced to be proline, based on analysis of its ^{13}C-NMR data, which showed five C signals of an amido C-atom, a C(α), and three CH_2, in addition to other signals corresponding to this structural moiety. The CD spectrum of **9** displayed a weak positive CE at 218 nm, a strong negative CE at 236 nm, in addition to a weak positive CE at 276 nm. The first two bands reflect the α-helical arrangement of the peptide chain, consistent with the computed molecular model. This structure was confirmed by the combined analysis of NOESY, COSY, TOCSY, HMQC, and HMBC spectra, which also led to the complete ^{1}H- and ^{13}C-NMR assignments (*Table 4*). The key NOESY correlations include H–C(8) to H–C(12′) (δ 7.28), and H–C(9) to H–C(12) (δ 7.40), similar to what is observed in the amphibine-B-type skeleton such as **7**, suggesting a similar conformation for both types of macrocyclic rings. The assignment for four amido C=O groups was achieved by the observation of the coupling of H–C(5) (δ 4.25) to C(4) (δ 167.5), H–C(9) (δ 5.74) to C(7) (δ 171.1), H–C(8) (δ 4.52) to C(1″) (δ 172.0), and CH_2(3′) (δ 3.21 and 2.93) to C(1′) (δ 170.1).

Compound **10** had a molecular formula $C_{29}H_{38}N_4O_4$, as deduced from its HR-EI-MS. Besides the skeletal signals indicated above, its ^{1}H-NMR spectrum ((D$_5$)pyridine) (*Table 4*) revealed characteristic signals of an isoleucine unit as the ring-incorporated amino acid residue. All the data confirmed the proposed structure **10**.

The isoleucine moiety of **10** was suggested by the ^{1}H-NMR signals of 2 Me groups, a *d* (δ 0.91) and a *t* (δ 0.70), and the N-terminal *N*-monomethylvaline unit by the signals of 2 Me groups (δ 0.76 and 0.71, 2*d*), H–C(2′) (δ 2.73, *d*), H–C(3′) (δ 1.80, *m*), and *Me*NH (δ 2.07). This last residue was confirmed as the N-terminal residue by displaying a base peak at *m*/*z* 86 (**e**) in the MS. The signals for H–C(1) and H–C(2) ((D$_5$)pyridine) were shifted downfield (δ 6.84 *vs.* 6.39) and upfield (δ 6.56 *vs.* 6.77), respectively, relative to the corresponding signals of **9** ($CDCl_3$). In addition, the coupling constant of H–C(2) and H–C(3) were very different (3.0 Hz in **10** *vs.* 10.4 Hz in **9**). These differences suggest that on varying the solvent, conformational changes of this 14-membered skeleton occurred. That the signal of C(1) appeared at relatively low field (δ 129.4 in **10** *vs.* 114.9 in **9**) supported this assumption. The CD spectrum of **10** displayed one strong negative CE band at 231 nm, consistent with the α-helical arrangement of the peptide chain.

To our knowledge, compounds **1** and **5**–**10** represent the first occurrence of such compounds, and they were named ramosines A–C and hemsines A–D, respectively, after their plant origins. Of these, hemsine B (**8**) has a structure very similar to that of lotusine B [13] in which the N-terminal amino acid residue is Me_2N–L–Leu instead of Me_2N–L–Ile in **8**. Ramosine C (**6**) possessing a 13′-OH group is the first derivative of this kind among the 14-membered cyclopeptide alkaloids.

Computer-modeling calculations[2]) revealed a common conformation for both amphibine-B-type and integerrine-type cyclopeptide ring systems. The models for these 14-membered bases, as represented by **6** and **9** in *Fig. 2*, revealed an endocyclic H-bond between amido C(7)=O and H–N(3) in addition to an exocyclic one between amido C(1′)=O and H–N(6). Inspection of these models indicated that their peptide torsion angles (ϕ and φ) conform to that of an α-helix. Thus, energy-minimized conformations of these cyclopeptide ring systems have their two endocyclic peptide residues locked by the styrylamine linkage in a conformation approximating that of an α-helix, which is extended further by the exocyclic peptides appendage. Close inspection of CD spectra of these cyclopeptides revealed a strong resemblance to the absorption pattern of α-helical poly-L-glutamic acid [14], which showed three CD bands above 185 nm, a positive CE near 192 nm, and two strongly overlapped negative CEs at *ca.* 208 and 222 nm. The 192 and 208 nm bands represent the split $\pi \rightarrow \pi^*$ transitions of the amide chromophore polarized perpendicularly and parallel,

respectively, to the helix axis, and the negative 222 nm band is due to the $n \rightarrow \pi^*$ transition. Among the 14-membered amphibine-B-type and integerrine-type analogs, the corresponding positive CE band appeared at *ca.* 214–221 nm, and the two negative CE bands showed up as a broad band at *ca.* 231–237 nm in all compounds, except for **2**, which showed split bands at 231 and 237 nm. The CD spectra also exhibited several positive CE bands above 300 nm, with one strong band near 354–356 nm, which could arise from the asymmetrically locked styrylamine moiety. For the 13-membered zizyphine-A-type cyclopeptides [3], the positive CE amide band was shifted to 228–232 nm and the negative CE bands appeared at *ca.* 248–252 and 262–267 nm, in addition to one strong positive CE band near 355–358 nm. In the 14-membered-ring system, conformational analysis revealed that the π-plane of the C(1)=C(2) bond is perpendicular to the π-plane of the benzene ring in the styrylamine moiety, while, in the 13-membered-ring system, both planes assume a *gauche* angle. The arrangement in the latter ring system would allow more resonance conjugation in the styrylamine moiety and the amide of the α-helix in the 13-membered cyclopeptide ring and account for the bathochromic shift of the CD curves with respect to that of 14-membered-ring system. The 14-methoxy substituent also contributed to this shift, which is reflected in the long-wave-length absorption bands in the UV spectra of this series of compounds.

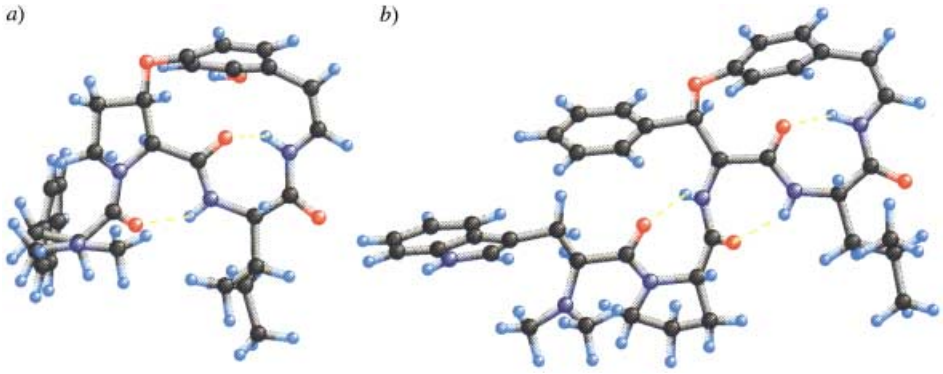

Fig. 2. *Molecular model of* a) *ramosine C* (**6** *and* b) *hemsine C* (**9**). Dashed lines indicate H-bonds.

It has been reported that certain 14-membered cyclopeptides such as *p*-phencyclopeptine possess the ionophore property, especially to sodium and magnesium ions, as established by CD measurements [15]. We utilized the same approach to investigate whether the above isolated alkaloids possess similar activity. However, no significant difference was observed in the CD spectra (MeOH) before and after adding selected salts, including sodium, magnesium, calcium, and potassium perchlorate. Other activities of the isolated 14-membered bases will be assayed after having accumulated larger amounts of material.

Experimental Part

General. For the recording of physical data, see [3][4]. TLC: silica gel; Me_2CO/toluene 3:7 (*A*), or 4:6 (*B*), both saturated with 25% NH_4OH soln. UV: λ_{max}(log ε) in nm. CD: λ ($\Delta\varepsilon$) in nm. IR: $\tilde{\nu}$ in cm^{-1}. MS: in *m*/*z* (rel. %).

Plant Material. Paliurus ramossismus, see [3]; *P. hemsleyanus*, see [7].

Extraction and Isolation from P. ramossismus. *Fr. 1* (45.3 mg) from the CPC [3] was chromatographed on a column (silica gel 230–400 mesh, 5 g; 0–0.5% MeOH/$CHCl_3$): **1** (18.6 mg). *Fr. 2* (95.4 mg) was separated by prep. TLC (silica gel, 20 × 20 cm, 0.5 mm; PhMe/Me_2CO 7:3, sat. with NH_4OH) to give seven subfractions. *Subfr. 2* (36.2 mg), *3* (47.4 mg), and *5* (22.8 mg) were chromatographed (silica gel, 70–230 mesh, each 4 g; 0–5% MeOH/$CHCl_3$): **2** (5.2 mg), **6** (5.3 mg) (from *Subfr. 2*), **3** (17.3 mg) (from *Subfr. 5*), and **5** (15 mg) (from *Subfr. 3*). *Fr. 6* (356 mg) was fractionated on a column (silica gel) to give nine subfractions [3]. *Subfr. 4* (66 mg) yielded **4** (39 mg) in addition to sativnine G (18 mg) upon separation by prep. TLC [3].

Ramosine A (= *Cyclo{-(3*S)-N^1-(N,N-*dimethyl*-L-*isoleucyl*)-*3-oxy*-L-*prolyl*-L-*isoleucyl*-*ψ*[*NH-CH=CH-(4,1-phenylene)*]-}; **1**). Colorless amorphous solid. R_f 0.44 (*A*). M.p. 55–56°. $[\alpha]_D^{26} = -125.0$ ($c = 0.76$, MeOH). UV (MeOH): 221 (4.52). CD ($c = 2.07 \cdot 10^{-5}$ M, MeOH): 306 (0), 282 (+1.13), 264 (+1.13), 256 (−0), 232 (−19.65). IR (KBr): 3394, 3315, 2965, 2933, 2876, 1690, 1625, 1505, 1482, 1229, 1171, 1118, 1078, 865. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS (pos.): 485 (65, $[M+H]^+$), 391 (25), 149 (100), 114 (91, **a**). HR-EI-MS: (484.3035 $[M+H]^+$, $C_{27}H_{40}N_4O_4$; calc. 484.3050).

Ramosine B (*Cyclo{-(3*S)-N^1-(N-*methyl*-L-*isoleucyl*-L-*phenylalanyl*)-*3-oxy*-L-*prolyl*-L-*isoleucyl*-*ψ*[*NH−CH=CH-(4,1-phenylene)*]-}; **5**). R_f 0.33 (*A*). $[\alpha]_D^{26} = -181.5$ ($c = 2.0$, MeCN). UV (MeOH): 216 (4.60), 233 (4.43), 329 (3.57). CD ($c = 1.62 \cdot 10^{-5}$ M, MeOH): 300 (+2.09), 285 (+0.79), 277 (+0.26), 261 (−1.46), 237 (−11.08). IR (KBr): 3394, 3325, 2960, 2930, 2870, 1690, 1620, 1500, 1480, 1222, 1170, 1110, 1079, 864. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: (51, $[M+H]^+$ 618), 344 (20), 100 (100, **b**).

Ramosine C (*Cyclo{-3-(3*S)-N^1-(N,N-*dimethyl*-L-*phenylalanyl*)-*3-oxy*-L-*prolyl*-L-*isoleucyl*-*ψ*[*NH−CH=CH-(3-hydroxy-4,1-phenylene]*-}; **6**). $[\alpha]_D^{26} = -39.0$ ($c = 1.0$, MeOH). UV (MeOH): 218 (4.75), 285 (3.98). UV (MeOH + NaOMe): 234 (4.69), 298 (3.98). CD ($c = 1.87 \cdot 10^{-5}$ M, MeOH): 306 (0), 286 (−6.99), 265 (0), 247 (−6.89), 238 (0), 226 (+23.44). IR (KBr): 3380, 3320, 2960, 2930, 2870, 1690, 1626, 1520, 1500, 1225, 1169, 1110, 1079, 862. ^{1}H- and ^{13}C-NMR: *Table 2*. FAB-MS (pos.): 535 (53, $[M+H]^+$), 307 (24), 154 (100), 148 (54, **c**). HR-FAB-MS (pos.): (535.2927 $[M+H]^+$, $C_{30}H_{39}N_4O_5^+$; calc. 535.2921).

Additional Data for Mucronine J (**2**). UV (MeOH): 219 (4.50). CD ($c = 2.07 \cdot 10^{-5}$ M, MeOH): 305 (0), 283 (+0.74), 267 (+0.69), 259 (0), 233 (−12.08).

Additional Data for Lotusine D (**3**). CD ($c = 1.98 \cdot 10^{-5}$ M, MeOH): 306 (0), 284 (sh, +1.31), 265 (+1.71), 256 (0), 235 (−15.27). HR-EI-MS: (504.2749 $[M+H]^+$, $C_{29}H_{36}N_4O_4^+$; calc. 504.2736).

Additional Data for Lotusine A (**4**). CD ($c = 1.95 \cdot 10^{-5}$ M, MeOH): 306 (0), 282 (sh, +1.06), 263 (+1.76), 252 (0), 234 (−13.64).

Extraction and Isolation from P. hemsleyanus. Most of the hot EtOH extract (686 g out of 800 g) of the ground, dry roots of *P. hemsleyanus* (14 kg) were triturated with dist. H_2O (60°, 2 × 2 l) and 5% AcOH, (3 × 1 l) in sequence. The acidic layer was adjusted to pH 9.0 with 25% ammonia soln. and then partitioned with $CHCl_3$ (3 × 2 l) to give the free bases (2.17 g) [3]. Part of this fraction (808 mg) was chromatographed (silica gel (230–400 mesh, 40 g), 0–2% ^{i}PrOH/cyclohexane): **9** (20.4 mg), **10** (7.1 mg), **7** (40.1 mg), and nuciferine [6] (8.0 mg). Part of the free-base portion (787 mg) was subjected to a medium-pressure chromatography (silica gel *60*, 0–7% MeOH/$CHCl_3$): **8** (10.7 mg).

Hemsine A (*Cyclo{-(3*S)-N^1-(N,N-*dimethyl*-L-*tryptophyl*)-*3-oxy*-L-*prolyl*-L-*isoleucyl*-*ψ*[*NH−CH=CH-(4,1-phenylene)*]-}; **7**). R_f 0.37 (*B*). $[\alpha]_D^{26} = -64.5$ ($c = 2.0$, MeOH). UV: 224 (4.96), 272 (4.30), 280 (4.30), 290 (4.21). CD: 310 (0), 274 (+1.65), 262 (0), 236 (−17.51), 223 (+4.60). IR: 3390, 3320, 2960, 2930, 2873, 1678, 1660, 1620, 1501, 1480, 1450, 1225, 1170, 1116, 1070, 862. ^{1}H- and ^{13}C-NMR: *Table 3*. HMBC ($CDCl_3$): H−C(1) → C(2), C(13), C(13′); H−C(3) → C(1); H−C(5) → C(4), C(7), C(15), C(16), C(18); H−C(6) → C(5), C(15); H−C(8) → C(9), C(20), C(21); H−C(12) → C(12′), C(13); H−C(13) → C(1), C(12′), C(13′); H−C(17) → C(15), C(16); H−C(18) → C(5), C(15), C(16); H−C(19) → C(8), C(9); H_α−C(20) → C(8), C(9), C(19); H−C(2′) → C(3′), C(4′), Me_2N; H−C(6′) → C(4′), C(5′), C(8′), C(10′); H−C(9′) → C(5′), C(7′); H−C(12′) → C(4′), C(5′), C(10′); Me_2N → C(2′). FAB-MS (pos.): (558 $[M+H]^+$, 43), 427 (31), 187 (100, **d**), 130 (17), 91 (6). HR-FAB-MS: 558.3049 ($[M+H]^+$, $C_{32}H_{40}N_5O_4^+$; calc. 558.3081).

Hemsine B (*Cyclo{-(3*S)-N^1-(N,N-*dimethyl*-L-*isoleucyl*-L-*phenylalanyl*)-*3-oxy*-L-*prolyl*-L-*isoleucyl*-*ψ*[*NH−CH=CH-(4,1-phenylene)*]-}; **8**). $[\alpha]_D^{26} = -124.0$ ($c = 1.0$, MeOH). UV: 217 (4.68), 257 (sh, 4.14), 285 (3.92). CD: 286 (+2.41), 264 (+2.41), 238 (−15.17), 220 (+4.37). IR: 3390, 3340, 2960, 2935, 2870, 1660, 1630, 1505, 1482, 1230, 1176, 1118, 1079, 862, 755. ^{1}H- and ^{13}C-NMR: *Table 3*. HMBC (CD_3OD): H−C(5) → C(4), C(15), C(16), C(18); H−C(8) → C(7), C(9); H−C(9) → C(7), C(8); H−C(16) → C(17); H−C(17) → C(15), C(16); H−C(18) → C(5), C(15), C(16); H_β−C(19) → C(8), C(9); H_α−C(20) → C(8), C(9); H−C(2′) → C(1′), C(4′), C(6′), Me_2N; H−C(4′) → C(5′); H−C(5′) → C(3′), C(4′); H−C(6′) → C(2′), C(3′), C(4′), Me_2N → C(2′);

H–C(2″) → C(1″), C(3″), C(4″); H–C(3″) → C(1″), C(2″), C(4″), C(5″) (C(9″)). FAB-MS (pos.): 632 (29, $[M+H]^+$), 501 (17), 307 (24), 289 (17), 154 (100), 137 (59), 114 (58, **a**), 91 (11).

Hemsine C (*Cyclo[-(βS)-N$^{\alpha}$-(*N,N-*dimethyl-*L*-tryptophyl-*L*-prolyl)-β-oxy-*L*-phenylalanyl-*L*-leucyl-ψ[NH–CH=CH-(4,1-phenylene)]-]*; **9**). R_f 0.52 (*B*). $[\alpha]_D^{26} = -107.0$ (*c* = 1.0, MeOH). UV: 214 (4.28), 280 (4.31). CD: 311 (–4.02), 296 (–2.76), 281 (–2.19), 231 (– 36.79). IR: 3398, 3300, 2980, 2930, 2890, 1680, 1650, 1620, 1501, 1480, 1225, 1185, 1110, 1068, 870, 740, 700. ^{1}H- and ^{13}C-NMR: *Table 4*. FAB-MS (pos.): 705 (72, $[M+H]^+$), 574 (47), 187 (100, **d**), 135 (8). HR-FAB-MS (pos.): (705.3762 $[M+H]^+$; $C_{41}H_{49}N_6O_5^+$; calc. 705.3764).

Hemsine D (*Cyclo[-(βS)-N$^{\alpha}$-(*N-*methyl-*L*-valyl)-β-oxy-*L*-phenylalanyl-*L*-isoleucyl-ψ[NH–CH=CH-(4,1-phenylene)]-]*; **10**). R_f 0.54 (*B*). $[\alpha]_D^{26} = -573.3$ (*c* = 0.75, $CHCl_3$). UV: 217 (4.70), 230 (3.66), 255 (3.40). CD: 276 (+ 3.35), 236 (–22.38), 218 (+ 3.16). IR: 3300, 3270, 2960, 2925, 2870, 1678, 1650, 1625, 1525, 1505, 1455, 1240, 1175, 1120, 1015, 860, 750, 710. ^{1}H- and ^{13}C-NMR: *Table 4*. FAB-MS (pos.): 507 (48, $[M+H]^+$), 422 (3), 224 (3), 135 (7), 91 (12), 86 (100, **e**). HR-EI-MS: 506.2888 (M^+, $C_{29}H_{38}N_4O_4^+$; calc. 506.2894).

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