Cyclopeptide Alkaloids from *Paliurus ramossisimus*

Hui-yi Lin,† Chung-Hsiung Chen, Bih-Jing You, Karin C. S. Chen Liu, and Shoei-Sheng Lee*

School of Pharmacy, College of Medicine, National Taiwan University, Taipei 100, Taiwan, Republic of China

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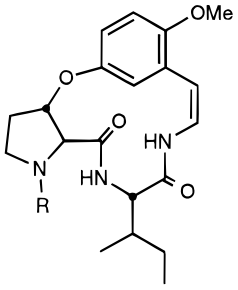
Seven zizyphine A-type cyclopeptide alkaloids were isolated from the roots of *Paliurus ramossisimus* by the combination of centrifugal partition chromatography and conventional separation methods. The novel structures of paliurines A−F (**1**−**6**) were characterized and established on the basis of MS and elaborate NMR spectral analyses. Terminal dipeptide stereochemistry was confirmed by correlation with the synthetic dipeptides via comparison of their ^{13}C NMR data.

Paliurus ramossisimus (Lour.) Poir (Rhamnaceae), a plant growing along the west coast of Taiwan Island, has been used as a pain reliever in the treatment of toothache and stomach ache. Previous investigations of its chemical constituents yielded the triterpenes ceanothic acid and betulinic acid as major components[1,2] and cyclopeptide alkaloids as very minor constituents. In the present paper we report the isolation and structural characterization of seven cyclopeptide alkaloids and the determination of stereochemistry for the terminal dipeptide residues.

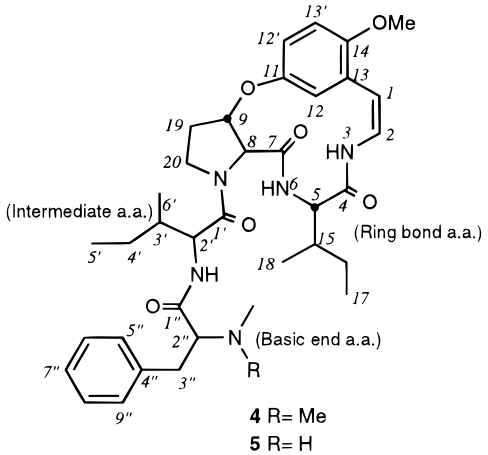
Results and Discussion

The methanolic extract of the roots was processed in a manner to facilitate the separation of alkaloids. As the cyclopeptide alkaloids are weak bases and less polar relative to alkaloids such as aporphines, the alkaloid-containing fraction usually mixes with other components having similar polarity. Thus, a focusing procedure, that is, passing this fraction through a Sephadex LH-20 column, was followed, which effectively separated the major alkaloid fraction. The components in this alkaloid fraction had very similar polarity and were separated previously by repeated column chromatography and preparative TLC. We found that separation could be facilitated by the combination of partition chromatography with conventional methods. We observed that the technique of applying acidic mobile phase would disturb the elution order of these components, not following their polarity as revealed on TLC plate. Thus, two components with different polarity to some extent were usually found to elute in the same fraction, allowing easy separation simply by column chromatography or TLC.

Using this approach, seven 13-membered cyclopeptide alkaloids (**1**−**7**) were isolated. These seven compounds possess common IR absorptions at 3285−3400 cm^{-1} (−CON*H*), 2780−2790 cm^{-1} (−NCH_3), 16800−1690 and 16300−1655 cm^{-1} (-*C*ONH), 1625 cm^{-1} for a *cis* 1,2-disubstituted olefin, and 12300−1240 cm^{-1} for aryl alkyl ether functions. UV showed absorption maxima at 265 and 317 nm, both typical of 13-membered zizyphine A-type cyclopeptide alkaloids,[3,4] which possess a 2,5-dioxystyrylamine moiety. Their CD spectra exhibited two negative Cotton effects around 264 and 324 nm, indicating the same 5*S*,8*S*,9*S* configuration at the 13-membered ring moiety present in zizyphine A, whose stereochemistry has been confirmed by total synthesis.[5] Their ^1H NMR spectra showed common signals for the 14-OMe (ca. δ 3.77); the protons H-1 (ca. δ 5.90, d), H-2 (ca. δ 6.93, dd), and H-3 (ca. δ 8.40, d), appearing as an AMX system,[6] and H-12 (ca. δ 6.65, d), H-12′ (ca. δ 6.77, dd) and H-13′ (ca. δ 6.86, d), appearing as an ABX system; the coupling pattern of H-8 (ca. δ 4.39, d) and H-9 (ca. δ 5.40, ddd or dt) specific for the (3*S*)-β-hydroxyproline unit; and, finally, two methyl signals (ca. δ 1.00, d; ca. δ 0.90, t) in the isoleucine unit. The coupling relationships of these protons were identified by a COSY-45 spectra. These data indicate compounds **1**−**7** to be zizyphine A-type 13-membered cyclopeptide alkaloids.[7] Further analysis of their ^{13}C NMR spectra revealed C-11 (s) and C-14 (s) near δ 151, C-1 (d) near δ 107, C-2 (d) near δ 122, and C-9 (d) in the β-hydroxyproline near δ 77.0. The mass spectra of these compounds, except that of **3**, revealed characteristic fragment ions at *m*/*z* 374,[7] which supported this type of structure having an isoleucine unit as the ring bond amino acid.



1 R= L-Phe-L-Ile(NMe_2)
2 R= L-Phe-L-Ile(NHMe)
3 R= L-Ile(NMe_2)
6 R= L-Phe(NMe_2)
7 R= L-Ile-L-Leu(NMe_2)



4 R= Me
5 R= H

* To whom correspondence should be addressed. Tel./Fax: 886-2-23916127. E-mail: shoeilee@ha.mc.ntu.edu.tw.
† Current address: Chinese Pharmaceutical Sciences and Department of Pharmacy, China Medical College, Taichung, Taiwan, Republic of China.

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Table 1. ^{1}H NMR Data for Compounds **1**−**7** in the Terminal Dipeptide Region (δ/ppm, J in Hz)[a] in $CDCl_3$

position	**1**[b]	**1**(CD_3OD)[b]	**2**[b]	**3**	**4**	**5**	**6**	**7**[b]
intermediate a.a	Phe	Phe	Phe		Ile	Ile		Ile
2′	5.01 br q (8.1)	4.95 dd (8.0, 6.9)	5.00 dt (8.2, 8.6)		4.53 t (8.5)	4.60 dd (8.6, 8.2)		4.55 dd (8.9, 8.5)
3′	2.93 dd (14.4, 8.3) 2.94 dd (14.4, 6.4)	3.04 dd (13.6, 6.9) 2.88 dd (13.6, 8.0)	2.95 m		1.70 m	1.74 m		1.77 m
4′					1.09 & 1.38	1.08 & 1.38		1.06 & 1.44
5′(9′)	7.05 m	7.21 br d (7.1)	7.04 m		0.83 t (7.5)	0.82 t (6.7)		0.84 t (7.5)
6′(8′)	7.24 m	7.26 t (7.1)	7.24 m		0.78 d (6.7)	0.81 d (6.5)		0.81 d (6.8)
7′	7.24 m	7.21 m	7.24 m					
2α′(N*H*)	7.05 d (8.8)		7.78 d (8.6)					7.41 d (8.9)
basic end a.a.	Ile(NMe_2)	Ile(NMe_2)	Ile(NHMe)	Ile(NMe_2)	Phe(NMe_2)	Phe(NHMe)	Phe(NMe_2)	Leu(NMe_2)
2″	2.53 d (5.5)	2.66 d (8.8)	2.81 d (4.6)	3.07 d (9.7)	4.30 dt (2.6, 9.7)	4.33 ddd (10.4, 8.8, 1.6)	3.42 dd (10.3, 3.4)	2.89 dd (8.4, 5.0)
3″	1.76 m	1.79 m	1.71 m	1.88 m	2.58 m	2.59 m	3.06 dd (13.0, 10.3) 2.91 dd (13.0, 3.4)	1.40 & 1.53
4″	1.12 & 1.44	1.07 & 1.57	1.12 & 1.40	1.10 & 1.58				1.10
5″(9″)	0.91 t (7.1)	0.88 t (7.3)	0.84 t (7.2)	0.87 t (7.3)	7.14 d (5.3)	7.19 br d (6.4)	7.04 dd (7.2, 1.2)	0.90 d (6.4)
6″(8″)	0.75 d (7.2)	0.77 (d, 6.7)	0.87 d (6.8)	0.71 d (6.5)	7.22 m	7.27 m	7.17 m	0.92 d (6.5)
7″					7.22 m	7.27 m	7.17 m	
NMe	2.20 s	2.12 s	2.33 s	2.33 s	2.29 s	2.25 s	2.39 s	2.24 s

[a] Signals without multiplicity were assigned from COSY-45 or HMQC spectra. [b] Data were assigned from analysis of COSY-45, NOED, HMQC, and HMBC spectra.

Compound **1** had molecular formula $C_{37}H_{51}N_5O_6$ as deduced from FABMS ($[M + H]^+$ at m/z 662) and analysis of its ^{13}C NMR data. The ^{1}H NMR spectrum of **1** ($CDCl_3$) (Table 1) revealed signals of two additional methyls from another isoleucine unit at δ 0.75 (d, J = 7.2 Hz) and 0.91 (t, J = 7.1 Hz) and five aryl protons belonging to a phenylalanine unit between δ 7.05 and δ 7.24 (m). Thus, **1** was composed of four amino acid residues (Phe, Ile, Ile, Pro) and a styrylamine unit. Of these, the ring amino acids were Ile and Pro as indicated above. In addition, the ^{1}H NMR spectrum revealed a six-proton singlet at δ 2.20, suggesting the terminal amino acid residue to be *N*,*N*-dimethylated. Its mass spectrum displayed the base peak at m/z 114, corresponding to a fragment ion typically obtained from β-cleavage of a *N*,*N*-dimethylated isoleucine unit. These data indicated the structure of **1** to be as shown.

Structure **1** was supported by NOED experiments. The critical NOEs included the common enhancement of N^3-H (δ 8.40, d) upon irradiation of N^6-H (δ 7.27, d) and H-12 (δ 6.65, d), respectively. No significant enhancement was observed between mutual irradiation of H-8 (δ 4.39, d) and H-9 (δ 5.40, dt). This observation also supported *trans*-relationship between H-8 and H-9. Irradiation of the *N*,*N*-dimethyl singlet enhanced a methyl doublet (δ 0.75, Me-6″), in addition to the signals at δ 2.53 (d, H-2″, α-H) and δ 1.76 (m, H-3″). This established *N*,*N*-dimethylisoleucine to be the terminal amino acid residue. An energy-minimized conformation of **1**, which accounts for the above NOED results, was obtained. In this conformation the proline plane adopts a perpendicular arrangement with the benzene ring of styrene, resulting in the closeness of H-9 and H-12. An exocyclic hydrogen bond extending between N^6-H and C-1′ keto group was observed, accounting for the nearness of H-8 and N^6-H.

Incorporating these ^{1}H NMR assignments (Table 1) from NOED and COSY-45 analysis, the chemical shifts of those proton-attached carbons were assigned directly from the analysis of a HETCOR spectrum, while those of the quaternary carbons were assigned from an HMBC spectral analysis (Table 2). The latter technique distinguished the signals of C-11 (δ 151.2) and C-14 (151.6) via observation of a three-bond coupling between C-14 and 14-OC*H*$_3$. For reference, the ^{1}H and ^{13}C NMR data of **1** measured in CD_3OD (Tables 1 and 2) were also assigned based on analysis of COSY-45, NOESY, and HMBC spectra.

Compound **2** had the molecular formula $C_{36}H_{49}N_5O_6$, deduced from analysis of its ^{13}C NMR data and FABMS ($[M + H]^+$ at m/z 648, 14 mass units lower than that of **1**). Its ^{1}H NMR spectrum was very similar to that of **1**, except for a three-proton *N*-Me singlet at δ 2.33 instead of a six-proton *N*Me_2 at δ 2.20 in **1**. Hence, **2** was likely to be the *N*-mono-demethylated analogue of **1**. The mass spectrum of **2** exhibited a base peak at m/z 100, which supported this conlusion. The complete ^{1}H and ^{13}C NMR data assignments of **2** (Tables 1 and 2) were made by analysis of its COSY-45, HETCOR, and COLOC spectra. Among these, the signals of amide carbons, C-4 (δ 167.1), C-7 (δ 169.9), C-1′ (δ 171.2), and C-1″ (δ 173.3), were distinguished by their coupling to H-3 (δ 8.40) (C-4), H-8 (δ 4.41) (C-7), H-3′ (δ 2.95) and H-2′ (δ 5.00) (C-1′), and H-2′a (CON*H*, δ 7.78) (C-1″). It is noted that the difference of *N*-substitution between **1** and **2** caused the shift change for β-carbon (C-3″, δ 34.4 in **1** vs. δ 38.3 in **2**) and amide carbon (C-1″, δ 171.5 in **1** vs δ 173.3 in **2**), due to γ-effect from extra *N*-methyl group in **1**.

Compound **3** had molecular formula $C_{28}H_{42}N_4O_5$, based on analysis of its ^{13}C NMR and FABMS data ($[M + H]^+$ at m/z 515). One more amino acid residue, in addition to the two (Ile and Pro) in the 13-membered ring moiety, was observed as evidenced by the presence of three amido carbonyl signals (δ 167.3, 170.5, and 170.9) in its ^{13}C NMR spectrum (Table 2). Besides the similar signals for those protons in the ring moiety as in **1**, its ^{1}H NMR spectrum also revealed signals for an *N*,*N*-dimethyl isoleucine residue, one methyl doublet (δ 0.71, H-6″), one methyl triplet (δ 0.87, H-5″), and a six-proton singlet (δ 2.33, NMe_2). The presence of this terminal amino acid was also supported by its mass spectrum, which showed the base peak at m/z 114. Based on these data, the structure of compound **3** was assigned as shown.

Compound **4** had the molecular formula $C_{37}H_{51}N_5O_6$, as deduced from FABMS and NMR data, the same as that of

Table 2. ^{13}C NMR Data for Compounds **1**−**7** in the Terminal Dipeptide Region[a] (δ/ppm, m[b]) in $CDCl_3$

position	**1**[c]	**1**[c].CD_3OD	**2**[c]	**3**	**4**	**5**[c]	**6**	**7**
intermediate a.a	Phe	Phe	Phe		Ile	Ile		Ile
1′	171.2 s	172.0 s	171.2 s		171.6 s	171.6 s		172.0 s
2′	51.0 d	53.1 d	51.1 d		53.8 d	53.8 d		53.9 d
3′	39.6 t	38.7 t	39.5 t		37.6 d	37.5 d		37.6 d
4′	135.6 s	137.8 s	135.7 s		24.5 t	24.5 t		24.7 t
5′(9′)	129.1 d	130.4 d	129.2 d		10.7 q	10.9 q		10.8 q
6′(8′)	128.8 d	129.7 d	128.8 d		15.2 q	15.3 q		15.4 q
7′	127.3 d	128.0 d	127.2 d					
basic end a.a.	Ile(NMe_2)	Ile(NMe_2)	Ile(NHMe)	Ile(NMe_2)	Phe(NMe_2)	Phe(NHMe)	Phe(NMe_2)	Leu(NMe_2)
1″	171.5 s	173.3 s	173.3 s	170.9 s	172.3 s	173.7 s	171.2 s	173.7 s
2″	74.4 d	74.0 d	69.9 d	68.3 d	71.0 d	66.1 d	68.0 d	67.7 d
3″	34.4 d	34.8 d	38.3 d	34.7 d	33.1 t	38.9 t	32.2 t	37.2 t
4″	26.9 t	26.6 t	25.3 t	25.3 t	139.7 s	137.3 s	138.2 s	26.0 d
5″(9″)	11.8 q	11.2 q	11.7q	15.1 q	129.1 d	129.0 d	128.9 d	23.3[d] q
6″(8″)	14.5 q	15.6 q	15.8 q	10.7 q	128.3 d	128.7 d	128.6 d	22.2[d] q
7″					126.1 d	126.9 d	126.5 d	
NMe	43.1 q	42.3 q	36.1 q	41.7 q	42.3 q	35.4 q	41.9 q	42.3 q

[a] A typical ^{13}C NMR data of the common ring nucleus, for example in **1**, are as follows: δ 106.7 (d, C-1), 121.6 (d, C-2), 167.0 (s, C-4), 60.3 (d, C-5), 169.8 (s, C-7), 64.8 (s, C-8), 76.6 (d, C-9), 151.2 (s, C-11), 111.4 (d, C-12), 117.7 (d, C-12′), 124.6 (s, C-13), 114.1 (d, C-13′), 151.6 (s, C-14), 35.6 (d, C-15), 24.9 (t, C-16), 11.8 (q, C-17), 16.3 (q, C-18), 32.4 (t, C-19), 46.4 (t, C-20). [b] Multiplicity was obtained from DEPT experiments. [c] Data were assigned from analysis of COSY-45, NOED, NOESY, HMQC, and HMBC spectra. [d] Both assignments could be interchanged.

1. Analysis of its ^1H and ^{13}C NMR spectra indicated that it contained the same amino acid residues (Ile × 2, Pro × 1, Phe × 1) as **1**. Because those signals from ring skeletons were similar for both compounds, it appeared that **4** was a structural isomer of **1**, the difference being in the sequence of the intermediate and terminal amino acid residues. The base peak at *m*/*z* 148 in the MS established *N*,*N*-dimethylphenylanaline as the terminal amino acid residue. By elimination, the intermediate amino acid residue was isoleucine, and the structure of **4** was determined as shown.

Compound **5** had the molecular formula $C_{36}H_{49}N_5O_6$, the same as **2** but 14 mass units less than **4**. The ^1H NMR spectrum of **5** was very similar to that of **4**, except for a three-proton *N*-Me singlet at δ 2.25 instead of a six-proton NMe_2 at δ 2.29 in **4**. Hence, **5** was likely to be the *N*-monodemethylated analogue of **4**. The mass spectrum exhibited a base peak at *m*/*z* 134, consistent with fragmentation typical of the terminal amino acid *N*-monomethyl phenylalanine. Thus, the structure of **5** was established as shown.

Compound **6** had the molecular formula $C_{31}H_{40}N_4O_5$. In addition to signals for the 13-membered nucleus including styrylamine, isoleucine, and β-hydroxyproline residues, its ^1H NMR spectrum displayed signals typical of an *N*,*N*-dimethyl phenylalanine residue: δ 7.04−7.17 (5H), 3.42 (1H, dd, H-2″), 3.06 (1H, dd) and 2.91 (1H, dd) (H−3″), and 2.39 (6H, s, NMe_2). The mass spectrum confirmed this suggestion, with a base peak at *m*/*z* 148. Consequently, the structure of **6** was determined as shown.

Compound **7** had the molecular formula $C_{34}H_{53}N_5O_6$. In addition to signals for the 13-membered ring nucleus, the ^1H NMR spectrum of **7** (Table 1) revealed signals of four methyls, three doublets (δ 0.92, 0.90 and 0.81), and one triplet (δ 0.84) and a six-proton singlet at δ 2.24. These data indicated that **7** was composed of four amino residues (Ile × 2, Leu, Pro) and a styrylamine unit. Of these, the ring amino acids were Ile and Pro as indicated above, while the intermediate and terminal amino acid residues were leucine and isoleucine, one of which was *N*,*N*-dimethylated. Upon irradiation of the six-proton singlet at δ 2.24 (NMe_2), the double doublet signal at δ 2.89 (J = 8.4 and 5.0 Hz) (α-H, H-2″) was enhanced, suggesting *N*,*N*-dimethylleucine to be the terminal amino acid residue. The mass spectrum, displaying a base peak at *m*/*z* 114, supported this assignment. Thus, the structure of **7** was determined to be as shown.

The stereochemistry in the ring nucleus of these seven compounds was deduced by the CD method as indicated above. Both intermediate and basic amino acids in other reported cyclopeptides were determined mostly to be the L- form by analysis of the products of acid hydrolysis.[7] We thought that NMR data (^1H and ^{13}C) could be distinct in this *N*-terminal dipeptide portion of different stereochemistry, and this could serve as a basis for determining the L or D forms of the amino acid residues. Thus, model compounds of the *N*-terminal dipeptides in cyclopeptide alkaloids of the zizyphine A type were prepared, and their NMR spectra were analyzed. These model dipeptides include four sets of diastereoisomers (both L,L and L,D forms), that is, Phe(OEt)-Leu(NMe_2), Ile(OMe)-Leu(NMe_2), Ile(OMe)-Phe(NMe_2), and Leu(OMe)-Phe(NMe_2); and two L,L-forms: Ile(OMe)-Ile(NMe_2) and Phe(OEt)-Ile(NMe_2). Starting from L-*N*-^{t}Boc-Ile, L-*N*-^{t}Boc-Phe, D-Phe (OEt), L- and D- Leu (*N*-Boc), these dipeptides were prepared by a common method, including three major steps, peptide bond formation via DCC coupling of an *N*-Boc amino acid and an amino acid ester,[8] followed by *N*-deprotection (removal of Boc, trifluoroacetic acid/H_2O = 3:1)[9] and *N*,*N*-dimethylation (HCHO/HCO_2H, Δ; or HCHO, H_2/Pd−C, MeOH).[10]

The ^1H NMR of these compounds showed the signal of α-H in the amino acid residue of the OR and NMe_2 terminal at δ 4.48−4.90 and 2.49−3.07, respectively, and the *N*,*N*-dimethyl singlet around δ 2.15−2.32. Comparison of the ^1H NMR of these diastereoisomers revealed no large differences among them. Thus ^1H NMR data were not suitable to distinguish them. Most of the ^{13}C NMR data of the synthetic dipeptides (Table 3) were assigned directly by analysis of the broad band decoupling and DEPT-135 spectra and also by intercorrelation, and consistency among these assignments were confirmed by 2D NMR techniques. For instance, L-Phe(OEt)-L-Leu(NMe_2), L-Phe(OEt)-D-Leu-(NMe_2), and L-Phe(OEt)-L-Ile(NMe_2) have common *C*-terminal residue [Phe(OEt)], by comparison the signals belonging to Phe(OEt) can be distinguished. The close carbonyl signals of ester and amide in L-Leu(OMe)-L-Phe-(NMe_2), L-Leu(OMe)-D-Phe(NMe_2), L-Ile(OMe)-L-Ile(NMe_2), and L-Phe(OEt)-L-Ile(NMe_2) were assigned by COLOC

Table 3. ^{13}C NMR Data of *N*,*N*-Dimethyl Dipeptide Pairs Phe(OEt)-Leu(NMe_2) and Leu(OMe)-Phe(NMe_2) in $CDCl_3$ (20 MHz)

	chemical shift					
	Phe(OEt)-Leu(NMe_2)[a]			Leu(OMe)-Phe(NMe_2)[b]		
position	LL[c]	LD[c]	$\Delta\delta$[d]	LL	LD	$\Delta\delta$
1	171.8 s	171.8 s	0.0	173.3 s	173.4 s	−0.1
2	52.6 d	52.8 d	−0.2	50.4 d	50.4 d	0.0
3	38.2 t	38.2 t	0.0	41.5 t	41.4 t	+0.1
4	136.4 s	136.4 s	0.0	24.8 d	24.8 d	0.0
5(9)	129.2 d	129.2 d	0.0	22.8 q	22.8 q	0.0
6(8)	128.4 d	128.5 d	−0.1	21.8 q	21.8 q	0.0
7	126.9 d	126.9 d	0.0			
1′	173.4 s	173.2 s	+0.2	172.2 s	172.1 s	+0.1
2′	67.7 d	67.1 d	**+0.6**[e]	71.0 d	70.6 d	**+0.4**
3′	37.3 t	36.3 t	**+1.0**	33.0 t	32.3 t	**+0.7**
4′	25.7 d	25.8 d	−0.1	139.8 s	140.1 s	**−0.3**
5′(9′)	23.4 q	23.3 q	+0.1	129.2 d	129.2 d	0.0
6′(8′)	22.0 q	22.1 q	−0.1	128.3 d	128.3 d	0.0
7′				126.0 d	126.0 d	0.0
NMe_2	42.3 q	42.0 q	**+0.3**	42.4 q	41.9 q	**+0.5**

[a] δ_{OEt} 61.2 (t) and 14.1 (q) in Phe(OEt)-Leu(NMe_2). [b] δ_{OMe} 52.0 (q) in L-Leu(OMe)-Phe(NMe_2). [c] L-Phe(OEt)-L-Leu(NMe_2) stands for *C*-terminal amino acid is L- form and *N*-terminal one is D-form. [d] $\Delta\delta$ (ppm) = $\delta_{LL} - \delta_{LD}$.

technique. For example, in L-Ile(OMe)-L-Ile(NMe_2) these two signals were assigned at δ 172.49 and 171.63, respectively, from the observation of three-bond coupling to ester OMe (δ 3.68). The ester or amide carbonyl signals in the other compounds were assigned by correlation. The resonance differences of the corresponding carbons between these diastereoisomers were observed and are listed in Table 3. Inspection of these data reveals significant shift differences between each pair of four sets of diastereoisomers for the chiral α-carbon (C-2′) and those around this chiral center (C-3′ and N*Me*$_2$) with different chirality. The shift difference ($\Delta\delta_{LL-LD}$) of C-2′ ranges from 0.2 to 0.8 ppm, that of C-3′ ranges from 0.6 to 1.5 ppm. The shift difference ($\Delta\delta_{LL-LD}$) of *N*,*N*-dimethyl carbons is relatively constant (ca. 0.30 ppm). Consequently, this method should be suitable for determining the stereochemistry of the terminal dipeptides in 13- and 14-cyclopeptide alkaloids.

The carbon chemical shifts of the *N*-terminal amino acid residue of compounds **1**, **2**, **4**, **5**, and **7** were very consistent with those of the corresponding amino acid residue in the prepared models of L−L dipeptides, but they were quite different from those of L−D ones. Hence, the intermediate and terminal basic amino acids are L forms.

Among these seven compounds, **3** was previously isolated from *Zizyphus sativa* (sativanine G[11]). However, we have now assigned its NMR data, which had not been reported. The other compounds, to our knowledge, represent the first natural occurrence of such products. We have, however, reported the conformation of paliurine B (**2**) based on the assumption of an all L-amino acid composition.[12] The trivial name paliurines **A**−**B** for compounds **1** and **2**, and paliurines **C**−**F** for compounds **4**−**7** were assigned based on their plant origin. The 14-membered cyclopeptide alkaloids have been reported to possess sedative effect.[13] Our preliminary study on such activity of these 13-membered bases (**1**−**7**), however, was not very consistent. Instead, we have indication that they possess immunostimulant activity, which deserves further exploration.

Experimental Section

General Experimental Procedures. Perkin-Elmer 1760-X infrared FT spectrometer (KBr); Hitachi 2000 UV (MeOH); JASCO J-710 spectropolarimeter (MeOH); JEOL JMX-HX110 mass spectrometer (FABMS: matrix, 4-nitrobenzyl alcohol; mass correlation, PEG600); Bruker AC-80 and AMX-400 NMR spectrometers in $CDCl_3$ (δ_H 7.24, δ_C 77.0) using Bruker standard pulse programs: in the HETCOR, HMQC, COLOC, and HMBC experiments, Δ = 1 s and J = 140, 140, 8, and 8 Hz, respectively, the correlation maps consisted of 512 × 1K data points per spectrum, each composed of 16 to 64 transients; Sanki CPC instrument, LLN type with 6 1000E cartridges (410 mL), using the upper and lower layer of the solvent system, $CHCl_3$−MeOH−6% HOAc (5:5:3), as mobile and stationary phases, respectively, 2.0 mL/min flow rate, 800 rpm rotation speed, about 40 kg/cm^2 pressure; Si gel TLC analysis [Me_2-CO−toluene (3: 7), saturated with 25% NH_4OH].

Plant Material. The roots of *Paliurus ramossisimus* for this study were collected in August 1989, along the west coast of Ho-Long, Miao Li, Taiwan. A voucher specimen (No. NTUPH-19890801) has been deposited in the School of Pharmacy, National Taiwan University.

Extraction and Isolation. The ground, dry roots (36 kg) were percolated with MeOH (150 L × 3). The MeOH extract (2.38 kg) was triturated with *n*-hexane (3 L × 3), and $CHCl_3$ (3 L × 3) to give fractions soluble in *n*-hexane (113 g) and $CHCl_3$ (396 g). The residue was suspended in H_2O (2 L) and further partitioned against EtOAc (3 L × 3) and *n*-BuOH (3 L × 3) to give fractions soluble in EtOAc (74 g), BuOH (378 g), and H_2O (708 g). The $CHCl_3$-soluble fraction was triturated with 1% NaOH (0.8 L × 4) to remove triterpenes such as betulinic acid and ceanothic acid.[2] The residue was then triturated with $CHCl_3$, and the soluble fraction (55 g) was dissolved in 95% EtOH and then passed through a Sephadex LH-20 column (1100 mL × 3, 95% EtOH) to give a fraction (16 g) containing cyclopeptide alkaloids. Part of this fraction (3 g) was separated on a CPC column using the conditions described above, monitored by Si gel TLC analysis, to give nine fractions. Fraction 4 yielded **2** (43 mg) after recrystallization from MeOH. Fraction 8 (35 mg) was separated by preparative TLC (0.5 mm) developed thrice with the TLC solvent indicated above to give **4** (23 mg). Fraction 5 (38 mg) was separated by preparative TLC (0.5 mm) developed thrice with Me_2CO−toluene (2:8), saturated with 25% NH_4OH, to give **2** (12 mg) and **5** (9 mg). Fraction 6 (356 mg) was chromatographed on a Si gel column (70 g, 70−230 mesh, 0−6% MeOH in $CHCl_3$) to give nine subfractions. Subfraction 4 (66 mg) was separated by preparative TLC (0.5 mm) developed with the TLC solvent indicated above to give **3** (18 mg) and a 14-membered cyclopeptide (39 mg). Subfraction 6 (101 mg) was separated by preparative TLC (1 mm and 0.5 mm) developed with Me_2CO−toluene (2:3 and 1:4), both saturated with 25% NH_4OH, respectively, to give **1** (34 mg) and **6** (15 mg) from the first preparative TLC and **7** (22 mg) from second one.

Paliurine A (1): amorphous powder; $[\alpha]^{26}_D$ −345.0° (*c* 1.0, MeOH); IR ν_{max} 3390, 3310, 2965, 2940, 2870, 1680, 1640, 1510, 1501, 1220, 1180, 1150, 1081, 879 cm^{-1}; UV λ_{max} (log ϵ) 216 (4.84), 269 (4.75), 318 (4.56) nm; CD (*c* 1.51 × 10^{-5} M) ($\Delta\epsilon$) 367 (+3.75), 356 (+4.16), 327 (−8.68), 313 (−10.01), 229 (+5.98); ^1H and ^{13}C NMR, see Tables 1 and 2 and Supporting Information; FABMS (positive) *m*/*z* (rel int) [M + H]$^+$ 662 (67), 628 (13), 374 (4, **a**), 114 (100, **b**); HRFABMS (positive) *m*/*z* [M + H]$^+$ 662.3901 (calcd for $C_{37}H_{52}N_5O_6$ 662.3918).

Paliurine B (2): colorless amorphous solid, mp 111−112 °C; $[\alpha]^{26}_D$ −391.3° (*c* 0.76, MeOH); IR ν_{max} 3328, 2963, 2934, 2876, 1642, 1510, 1455, 1220, 1183, 1089, 1036, 881, 799, 756, 701 cm^{-1}; UV λ_{max} (log ϵ) 216 (4.81), 268 (4.71), 319 (4.56) nm; CD (*c* 1.54 × 10^{-5} M) ($\Delta\epsilon$) 356 (+4.71), 314 (−10.84), 267 (−26.49), 252 (−22.48), 228 (+5.57); ^1H and ^{13}C NMR, see Tables 1 and 2 and Supporting Information; FABMS (positive) *m*/*z* (rel int) [M + H]$^+$ 648 (51), 614 (14), 374 (15, **a**), 346 (12), 100 (100, **c**); HRFABMS (positive) *m*/*z* [M + H]$^+$ 648.3754 (calcd for $C_{36}H_{50}N_5O_6$ 648.3761).

Sativnine G (3): colorless amorphous solid, mp 93−94 °C; $[\alpha]^{26}_D$ −327.0° (*c* 0.85, MeOH); IR ν_{max} 3334, 2964, 2935, 2876, 1674, 1642, 1510, 1463, 1224, 1183, 1111, 1092, 863, 757, 695 cm^{-1}; UV λ_{max} (log ϵ) 217 (4.66), 230 (4.51), 269 (4.65), 320 (4.47) nm; CD (*c* 1.95 × 10^{-5} M) ($\Delta\epsilon$) 356 (+5.75), 340 (0), 323

(−7.93), 315 (−8.15), 267 (−22.92), 250 (−15.31), 237 (0), 232 (+5.16), 225 (0), 217 (−8.02); ^{1}H and ^{13}C NMR, see Tables 1 and 2 and Supporting Information; FABMS (positive) *m/z* (rel int) $[M + H]^+$ 515 (22), 307 (58), 289 (31), 114 (100, **b**); HRFABMS (positive) *m/z* $[M + H]^+$ 515.3246 (calcd for $C_{28}H_{43}N_4O_5$ 515.3234).

Paliurine C (4): colorless amorphous solid; $[\alpha]^{26}_D$ −311.0° (*c* 1.00, MeCN); IR ν_{max} 3395, 2960, 2930, 2850, 1690, 1640, 1500, 1462, 1260, 1220, 1180, 1090, 1037, 880, 770, 700 cm^{-1}; UV λ_{max} (log ϵ) 214 (4.80), 268 (4.62), 318 (4.48) nm; CD (*c* 1.51 × 10^{-5} M) ($\Delta\epsilon$) 355 (+7.93), 315 (−5.52), 262 (−17.89), 249 (−13.36), 232 (4.12), 224 (0), 219 (−1.25); ^{1}H and ^{13}C NMR, see Tables 1 and 2 and Supporting Information; FABMS (positive) *m/z* (rel int) $[M + H]^+$ 662 (34), 570 (7), 374 (2, **a**), 289 (5), 148 (100, **d**).

Paliurine D (5): $[\alpha]^{26}_D$ −164.0° (*c* 1.00, MeCN); IR ν_{max} 3400, 3380, 2960, 2920, 2850, 1690, 1640, 1505, 1456, 1220, 1180, 1035, 890, 770, 700 cm^{-1}; UV λ_{max} (log ϵ) 216 (4.56), 234 (4.31), 269 (4.34), 318 (4.13) nm; CD (*c* 1.55 × 10^{-5} M) ($\Delta\epsilon$) 358 (+5.75), 336 (0), 313 (−4.88), 267 (−14.34), 248 (−11.86); ^{1}H and ^{13}C NMR, see Tables 1 and 2 and Supporting Information; FABMS (positive) *m/z* (rel int) $[M + H]^+$ 648 (69), 374 (20, **a**), 307 (30), 134 (100, **e**).

Paliurine E (6): $[\alpha]^{26}_D$ −382.3° (*c* 0.94, MeCN); IR ν_{max} 3390, 3345, 2960, 2930, 2870, 1670, 1638, 1502, 1460, 1415, 1380, 1290, 1260, 1220, 1180, 1102, 1078, 1055, 1040, 878, 810, 790, 750, 700 cm^{-1}; UV λ_{max} (log ϵ) 216 (4.82), 269 (4.70), 318 (4.55) nm; CD (*c* 1.82 × 10^{-5} M) ($\Delta\epsilon$) 356 (+5.69), 267 (−24.04), 240 (0), 231 (+14.11), 214 (+4.27); ^{1}H and ^{13}C NMR, see Tables 1 and 2 and Supporting Information; FABMS (positive) *m/z* (rel int) $[M + H]^+$ 549 (100), 374 (2, **a**), 307 (29), 154 (88), 148 (99, **d**), 136 (60); HRFABMS (positive) *m/z* $[M + H]^+$ 549.3112 (calcd for $C_{31}H_{41}N_4O_5$ 549.3077).

Paliurine F (7): $[\alpha]^{26}_D$ −323.0° (*c* 1.00, MeCN); IR ν_{max} 3400, 3330, 2955, 2930, 2860, 1690, 1630, 1505, 1435, 1365, 1262, 1221, 1180, 1040, 880, 820, 770 cm^{-1}; UV λ_{max} (log ϵ) 217 (4.62), 269 (4.56), 318 (4.38) nm; CD (*c* 1.59 × 10^{-5} M) ($\Delta\epsilon$) 355 (+7.42), 268 (−19.62), 262 (−20.27), 249 (−14.37), 231 (0), 219 (−9.13); ^{1}H and ^{13}C NMR ($CDCl_3$), see Tables 1 and 2 and Supporting Information; major NOEs: 14-OMe to H-13′ and H-1, H-3 to H-2, H-5 and H-12, H-5 to H-3, H-15 and H-18, H-6 to H-8, H-9 to H-12, H-19β and H-20β, H-12 to H-1, H-3 and H-9, H-2α′ (amide-H) to H-2′, H-2″ and N*Me*$_2$, N*Me*$_2$ to H-2α′, H-2″ and H-3″; key HMBC data: H-3 (δ 8.44) and H-5 (δ 4.26) to C-4 (δ 167.1), H-12 (δ 6.68), H-12′ (δ 6.79) and 14-OMe (δ 3.77) to C-14 (δ 151.5), H-13′ (δ 6.87) to 11 (δ 151.1), H-6 (δ 7.19) to C-7 (δ 170.3), H-2′ (δ 4.55) to C-1′ (δ 171.8), H-2″ (δ 2.89) and H-3″ (δ 1.53 and 1.40) to C-1″ (δ 173.1); FABMS (positive) *m/z* (rel int) $[M + H]^+$ 628 (40), 374 (2, **a**), 255 (4), 165 (2), 114 (100, **f**).

General Method for Preparation of *N,N*-Dimethyl Dipeptides. A representative method for preparing L-Ile-(OMe)-L-Phe(*N*-Me$_2$) via three-step reaction is described below.

1. Preparation of L-Ile (OMe) from L-Ile (*N*-^{t}Boc). To a methanolic solution (30 mL) of L-Ile (*N*-^{t}Boc) (3.30 g, 14.3 mmol) in a 250-mL round-bottom flask was added ethereal diazomethane freshly prepared from diazald (6.13 g). The flask was then screw-tightened and kept at 4 °C for overnight. The solution was evaporated to give a residue (3.25 g, 98.5%), which was essentially pure by TLC. Without further purification, part of the residue (600 mg, 2.44 mmol) in a 10-mL flask was mixed with trifluoroacetic acid (1.5 mL) and H_2O (0.5 mL), and the suspension was stirred at room temperature for 4 h. After evaporation under reduced pressure, the residue was partitioned between $CHCl_3$ (30 mL) and 5% ammonia water (20 mL × 3). The $CHCl_3$ layer was dried over $MgSO_4$ and evaporated to give a colorless viscous residue, L-Ile(OMe) (277 mg, 78% yield), ^{1}H NMR ($CDCl_3$) δ_{OMe} 3.65.

2. Preparation of L-Ile(OMe)-L-Phe(*N*-^{t}Boc). To the solution of L-Ile(OMe) (110 mg, 0.76 mmol) and L-Phe(*N*-^{t}Boc) (201 mg, 0.76 mmol) in CH_2Cl_2 (10 mL) and pyridine (five drops) in a 10-mL round-bottom flask was added dropwise the solution of *N,N*′-dicyclohexyl-carbodiimide (DCC) (234 mg, 1.14 mmol) in CH_2Cl_2 (5 mL) in an ice bath and stirred for additional 30 min and then at room temperature for 4 h. The excess DCC was destroyed by few drops of acetic acid, and the precipitate (*N,N*-dicylohexylurea, DCU) was removed by filtration. After evaporation, the residue was flash chromatographed over Si gel (17 g, 230−400 mesh) elution with 1 to 5% of Me_2-CO in toluene to give the product L-Ile(OMe)-L-Phe(*N*-^{t}Boc) (232 mg, 78% yield): ^{1}H NMR δ ($CDCl_3$) 3.65 (s, OMe), 4.95 (s, N*H*BOc), 1.39 (s, Boc-Me).

3. Preparation of L-Ile(OMe)-L-Phe(NMe$_2$). In a 25-mL round-bottom flask, l-Ile(OMe)-L-Phe(*N*-^{t}Boc) was mixed with trifluoroacetic acid (4.5 mL) and H_2O (1.5 mL), and the suspension was stirred at room temperature for 4 h. After evaporation under reduced pressure, the residue, which showed a single spot in TLC analysis without further purification, was *N,N*-dimethylated under catalytic conditions (10% Pd/C, 50 mg; H_2, 1 atm; EtOH 5 mL, HCHO 0.5 mL, overnight). The resultant suspension was filtered through a Celite cake, and the filtrate was condensed. The residue was then partitioned between $CHCl_3$ (20 mL) and 1% ammonia water (20 mL × 3). The $CHCl_3$ layer was dried over $MgSO_4$ and evaporated to give the crude product (222 mg), which was purified via a Si gel flash column (9 g, 230−400 mesh) eluted with 0 to 0.5% MeOH in $CHCl_3$ to give the product, L-Ile(OMe)-L-Phe(NMe$_2$) (203 mg, 91.5% yield).

The other dipeptides were prepared from commercially available (Sigma Co.) *N*- or *O*-protected amino acids, L-Phe-(OEt), D- and L-Phe(NBoc), L- and D-Leu(NBoc), and L-Ile(OMe) in a similar manner. The physical data of the compounds follow.

L-Phe(OEt)-L-Leu(NMe$_2$): ^{1}H NMR δ ($CDCl_3$, 80 MHz) 7.20 (6H, m, ϕ-H and amide-H), 4.82 (1H, dt, *J* = 6.0, 7.8 Hz, H-2), 3.11 (1H, dd, *J* = 11.6, 7.8 Hz, H-3a), 3.03 (1H, *J* = 11.6, 6.0 Hz, H-3b), 2.78 (1H, dd, *J* = 7.4, 6.1 Hz, H-2′), 2.15 (6H, s, NMe$_2$), 0.85 (3H, d, *J* = 6.0 Hz) and 0.83 (3H, d, *J* = 6.0 Hz) (H-5 and H-6), 4.14 (2H, q, *J* = 7.1 Hz, OC*H*$_2$CH$_3$), 1.35 (3H, t, *J* = 7.1 Hz, OCH$_2$C*H*$_3$); ^{13}C NMR data, see Table 3; EIMS *m/z* (rel int) $[M]^+$ 334 (2), 114 (100).

L-Phe(OEt)-D-Leu(NMe$_2$): ^{1}H NMR δ ($CDCl_3$, 80 MHz) 7.19 (6H, m, ϕ-H and amide-H), 4.81 (1H, dt, *J* = 6.6, 8.2 Hz, H-2), 3.12 (1H, m, H-3a), 3.04 (1H, m, H-3b), 2.82 (1H, dd, *J* = 8.1, 5.2 Hz, H-2′), 2.17 (6H, s, NMe$_2$), 0.89 (3H, d, *J* = 5.7 Hz) and 0.86 (3H, d, *J* = 6.1 Hz) (H-5 and H-6), 4.14 (2H, q, *J* = 7.1 Hz, OC*H*$_2$CH$_3$), 1.35 (3H, t, *J* = 7.1 Hz, OCH$_2$C*H*$_3$); ^{13}C NMR data, see Table 3; EIMS *m/z* (rel int) $[M]^+$ 334 (3), 115 (3), 114 (100).

L-Ile(OMe)-L-Leu(NMe$_2$): ^{1}H NMR δ ($CDCl_3$, 80 MHz) 7.29 (1H, d, *J* = 9.0 Hz, amide-H), 4.50 (1H, dd, *J* = 9.0, 4.9 Hz, H-2), 3.68 (3H, s, OCH_3), 2.82 (1H, dd, *J* = 7.9, 5.6 Hz, H-2′), 2.24 (6H, s, NMe$_2$), 0.88 (9H, d, *J* = 6.1 Hz, H-6, H-5′ and H-6′), 0.84 (3H, t, *J* = 6.1 Hz, H-5); ^{13}C NMR data, see Supporting Information; EIMS *m/z* (rel int) $[M]^+$ 286 (1), 215 (7), 114 (100).

L-Ile(OMe)-D-Leu(NMe$_2$): ^{1}H NMR δ ($CDCl_3$, 80 MHz) 7.37 (1H, d, *J* = 9.0 Hz, amide-H), 4.50 (1H, dd, *J* = 9.0, 4.8 Hz, H-2), 3.67 (3H, s, OCH_3), 2.90 (1H, dd, *J* = 8.0, 5.0, Hz, H-2′), 2.25 (6H, s, NMe$_2$), 0.92−0.83 (12H, m, H-5, H-6, H-5′, and H-6′); ^{13}C NMR data, see Supporting Information; EIMS *m/z* (rel int) $[M]^+$ 286 (2), 114 (100).

L-Ile(OMe)-L-Phe(NMe$_2$): ^{1}H NMR δ ($CDCl_3$, 80 MHz) 7.62 (1H, d, *J* = 8.8 Hz, amide-H), 7.18 (5H, m, ϕ-H), 4.45 (1H, dd, *J* = 8.8, 6.0 Hz, H-2), 3.62 (3H, s, OCH_3), 3.50−2.77 (3H, m, H-2′, H-3′a, and 3′b), 2.27 (6H, s, NMe$_2$), 0.86 (3H, t, *J* = 5.3 Hz, H-5), 0.82 (3H, d, *J* = 6.7 Hz, H-6); ^{13}C NMR data, see Supporting Information; EIMS *m/z* (rel int) $[M]^+$ 320 (0.4), 230 (7), 229 (35), 148 (100).

L-Ile(OMe)-D-Phe(NMe$_2$): ^{1}H NMR δ ($CDCl_3$, 200 MHz) 7.23−7.11 (6H, m, ϕ-H and amide-H), 4.45 (1H, dd, *J* = 8.9, 5.0 Hz, H-2), 3.69 (3H, s, OCH_3), 3.46 (1H, dd, *J* = 7.8, 5.3 Hz, H-2′), 3.17 (1H, dd, *J* = 13.6, 7.8 Hz) and 2.92 (1H, dd, *J* = 13.6, 5.3 Hz) (H-3′a and 3′b), 2.39 (6H, s, NMe$_2$), 0.83 (3H, t, *J* = 7.2 Hz, H-5), 0.75 (3H, d, *J* = 6.9 Hz, H-6); and ^{13}C NMR data, see Supporting Information; EIMS *m/z* (rel int) $[M]^+$ 320 (0.5), 230 (6), 229 (33), 147 (7), 148 (100).

L-Leu(OMe)-L-Phe(NMe$_2$): ^{1}H NMR δ ($CDCl_3$, 80 MHz) 7.20 (6H, m, ϕ-H and amide-H), 4.52 (1H, dt, *J* = 5.6, 8.6 Hz, H-2), 3.65 (3H, s, OCH_3), 3.40−2.75 (3H, m, H-2′, H-3′a, and H-3′b), 2.29 (6H, s, NMe$_2$), 0.89 (6H, d, *J* = 5.0 Hz, H-5 and

H-6); ^{13}C NMR data, see Table 3; EIMS *m*/*z* (rel int) $[M]^+$ 320 (0.4), 230 (2), 229 (24), 149 (5), 148 (100).

L-Leu(OMe)-D-Phe(NMe_2): ^{1}H NMR δ ($CDCl_3$, 80 MHz) 7.21 (6H, m, ϕ-H and amide-H), 4.53 (1H, dt, J = 5.8, 9.3 Hz, H-2), 3.68 (3H, s, OCH_3), 3.40−2.77 (3H, m, H-2′, H-3′a, and H-3′b), 2.32 (6H, s, NMe_2), 0.85 (6H, d, J = 5.1 Hz, H-5 and H-6); ^{13}C NMR see Table 3; EIMS *m*/*z* (rel int) $[M]^+$ 320 (0.6), 230 (2), 229 (25), 149 (6), 148 (100).

L-Ile(OMe)-L-Ile(NMe_2): ^{1}H NMR δ ($CDCl_3$, 400 MHz) 6.93 (1H, d, J = 8.4 Hz, amide-H), 4.55 (1H, dd, J = 8.4, 4.8 Hz, H-2), 3.69 (3H, s, OCH_3), 2.55 (1H, d, J = 5.2 Hz, H-2′), 2.24 (6H, s, NMe_2), 0.91−0.86 (12H, m, H-5, H-5′, H-6, and H-6′); ^{13}C NMR δ ($CDCl_3$, 20 MHz) 172.5 (s, C-1), 171.6 (s, C-1′), 74.6 (d, C-2′), 56.1 (d, C-2), 51.7 (q, OCH_3), 43.2 (q, NMe_2), 37.6 (d, C-3), 34.5 (d, C-3′), 27.0 (t, C-4′), 25.2 (t, C-4), 15.8 (q, C-6), 14.6 (q, C-6′), 11.9 (q, C-5′), 11.4 (q, C-5); key COLOC data (100 MHz) C-1 (δ 172.5) to OCH_3 (δ 3.69); EIMS *m*/*z* (rel int) $[M]^+$ 286 (28), 275 (6), 230 (28), 229 (94), 115 (94), 114 (100).

L-Phe(OEt)-L-Ile(NMe_2): ^{1}H NMR δ ($CDCl_3$, 400 MHz) 7.28−7.21 (5H, m, ϕ-H), 6.76 (1H, d, J = 8.6 Hz, amide-H), 4.91 (1H, dt, J = 5.6, 8.6 Hz, H-2), 3.19 (1H, dd, J = 14.1, 5.6 Hz, H-3a), 3.01 (1H, dd, J = 14.1, 8.6 Hz, H-3b), 2.57 (1H, d, J = 4.7 Hz, H-2′), 2.21 (6H, s, NMe_2), 4.16 (2H, q, J = 7.1 Hz, O$CH_2$$CH_3$), 1.23 (3H, t, J = 7.1 Hz, OCH_2CH_3), 0.86 (3H, t, J = 7.3 Hz, H-5′), 0.63 (3H, t, J = 6.8 Hz, H-6′); ^{13}C NMR δ ($CDCl_3$, 20 MHz) 171.9 (s, C-1), 171.5 (s, C-1′), 136.4 (s, C-4), 129.1 (d, C-5 and C-9), 128.5 (d, C-6 and C-8), 126.9 (d, C-7), 74.5 (d, C-2′), 61.2 (t, O$CH_2$$CH_3$), 52.6 (d, C-2), 43.1 (q, NMe_2), 34.3 (d, C-3′), 26.8 (t, C-4′), 14.2 (q, C-6′), 14.1 (q, O$CH_2$$CH_3$), 11.9 (q, C-5′); key COLOC data (100 MHz) C-1 (δ 171.9) to H-2 (δ 4.91), H-3 (δ 3.01) and O$CH_2$$CH_3$ (δ 4.16); EIMS *m*/*z* (rel int) $[M]^+$ 334 (0.02), 115 (13), 114 (100).

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Supporting Information Available: HMBC and NOEs of paliurine A, key mass fragments, ^{1}H and ^{13}C NMR data in the ring nucleus, ^{13}C NMR of paliurines D and G versus synthetic dipeptides.

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