Two New Cycloheptapeptides from *Psammosilene tunicoides*

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Two new cycloheptapeptides, tunicyclins F and G, were isolated from the roots of *Psammosilene tunicoides*. Their chemical structures were elucidated by extensive analysis of 1D- and 2D-NMR, as well as HR-ESI-MS data. Tunicyclin G contains an unusual α,β-dehydrotryptophan (Δ^Z-Trp) residue, which mainly occurs in the cyclic peptides from marine sponge and bacteria.

Introduction. – Continuing our investigation on the chemical constituents of the root of *Psammosilene tunicoides* W. C. WU et C. Y. WU (Caryophyllaceace), a well-known medicinal herb used as anodyne and haemastatic agent in southwest China [1–4], we have previously reported five new cyclic peptides, tunicyclins A–E, and a known cyclic peptide, psammosilenin B [5–7]. Further studies on this plant led to the isolation of two new cycloheptapeptides, tunicyclins F and G (*Fig. 1*). Tunicyclin G contains an unusual α,β-dehydrotryptophan (Δ^Z-Trp) residue, which mainly occurs in the cyclic peptides from marine sponge and bacteria [8][9]. Here, we describe the isolation and structure elucidation of these two cyclopeptides.

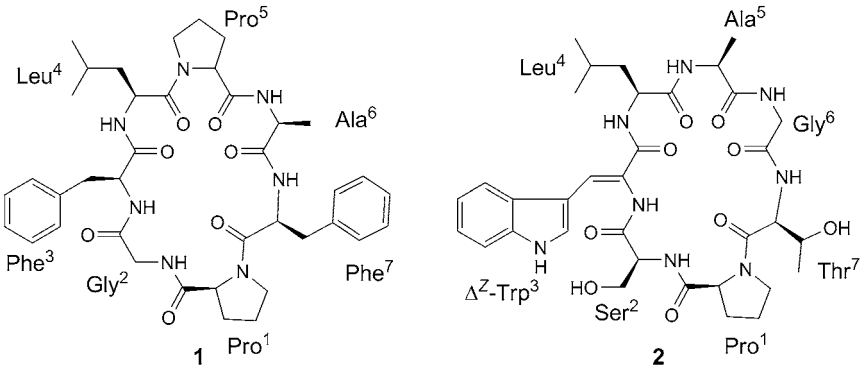

Fig. 1. *The chemical structures of* **1** *and* **2**

Results and Discussion. – Compound **1** was isolated as colorless solid ($[\alpha]_D^{20} = -74.6$, $c = 0.135$, MeOH). The molecular formula was established as $C_{39}H_{51}N_7O_7$ by

HR-ESI-MS (positive-ion mode; $[M+H]^+$ peak at m/z 730.3927; calc. 730.3923). The presence of seven amide CO resonances (δ(C) 173.0, 172.9, 172.7, 172.6, 172.4, 171.8, and 169.5), together with seven α-amino acid C-atom resonances (δ(C) 63.0, 61.5, 55.3, 53.4, 52.9, 52.7, and 43.8) in the ^{13}C-NMR spectrum (*Table 1*), and five amide H-atom signals (δ(H) 10.20, 9.03, 8.81, 8.69, and 8.38) in the ^{1}H-NMR spectrum indicated that **1** is a typical cycloheptapeptide. In addition, signals of three Me groups, ten CH_2 groups, among them three bound to N, one CH group, and two Ph groups were also detected in the 1D-NMR spectra of **1**. From ^{1}H,^{1}H-COSY and TOCSY experiments, five amino acid spin systems of Pro, Gly, Leu, Pro, and Ala were unambiguously determined (*Fig. 2*) [10][11]. Two Phe residues were undoubtedly determined based on the HMBC correlations (*Fig. 2*) between the β-H-atoms of the two Phe residues (δ(H) 3.69 (Phe^a-H(β_a)), 3.26 (Phe^a-H(β_b)); and 3.65 (Phe^b-H(β_a)), 3.35 (Phe^b-H(β_b))), and the C(1′) resonances (δ(C) 139.6 (Phe^a) and 137.4 (Phe^b)) of two Ph groups, respectively. The assignments of the protonated C-atoms were obtained from the HMQC spectrum, in

Table 1. *^{1}H- and ^{13}C-NMR Data of* **1** (at 500 and 125 MHz, resp., in C_5D_5N, J in Hz, δ in ppm)

Residue	δ(H)	δ(C)	Residue	δ(H)	δ(C)
Pro[1]			H_b–C(β)	1.52–1.58 (*m*)	
CO		172.7	H–C(γ)	2.18–2.25 (*m*)	25.3
H–C(α)	4.55 (*t*, *J* = 8.0)	63.0	Me(δ_a)	0.78 (*d*, *J* = 6.5)	23.6
H_a–C(β)	2.23–2.28 (*m*)	29.3	Me(δ_b)	1.15 (*d*, *J* = 6.5)	21.5
H_b–C(β)	2.08–2.15 (*m*)		*Pro*[5]		
H_a–C(γ)	2.01–2.08 (*m*)	26.1	CO		172.4
H_b–C(γ)	1.78–1.84 (*m*)		H–C(α)	4.77–4.83 (*m*)	61.5
H_a–C(δ)	3.52–3.60 (*m*)	47.8	H_a–C(β)	2.51–2.57 (*m*)	32.9
H_b–C(δ)	3.52–3.60 (*m*)		H_b–C(β)	2. 26–2.34 (*m*)	
Gly[2]			H_a–C(γ)	1.80–1.88 (*m*)	22.7
CO		169.5	H_b–C(γ)	1.73–1.80 (*m*)	
NH	9.03 (*t*, *J* = 6.0)		H_a–C(δ)	3.62–3.68 (*m*)	47.7
H_a–C(α)	4.44 (*dd*, *J* = 7.0, 12.0)	43.8	H_b–C(δ)	3.62–3.68 (*m*)	
H_b–C(α)	4.30 (*dd*, *J* = 6.0, 12.0)		*Ala*[6]		
Phe[3]			CO		173.0
CO		172.6	NH	8.81 (*d*, *J* = 9.0)	
NH	8.69 (*d*, *J* = 7.0)		H–C(α)	4.83–4.89 (*m*)	53.4
H–C(α)	5.31–5.37 (*m*)	55.3	Me(β)	1.54 (*d*, *J* = 7.0)	18.8
H_a–C(β)	3.69 (*dd*, *J* = 9.3, 14.0)	37.5	*Phe*[7]		
H_b–C(β)	3.26 (*dd*, *J* = 3.5, 14.0)		CO		171.8
C(1′)		139.6	NH	8.38 (*d*, *J* = 8.5)	
H–C(2′)	7.65 (*d*, *J* = 7.5)	129.8	H–C(α)	5.44–5.50 (*m*)	52.7
H–C(3′)	7.36 (*t*, *J* = 7.5)	128.9	H_a–C(β)	3.62–3.68 (*m*)	38.9
H–C(4′)	7.23 (*t*, *J* = 7.5)	126.5	H_b–C(β)	3.35 (*dd*, *J* = 8.4, 13.5)	
H–C(5′)	7.36 (*t*, *J* = 7.5)	128.9	C(1′)		137.4
H–C(6′)	7.65 (*d*, *J* = 7.5)	129.8	H–C(2′)	7.71 (*d*, *J* = 7.5)	130.9
Leu[4]			H–C(3′)	7.35 (*t*, *J* = 7.5)	128.8
CO		172.9	H–C(4′)	7.27 (*t*, *J* = 7.5)	127.2
NH	10.20 (*d*, *J* = 5.0)		H–C(5′)	7.35 (*t*, *J* = 7.5)	128.8
H–C(α)	4.77–4.83 (*m*)	52.9	H–C(6′)	7.71 (*d*, *J* = 7.5)	130.9
H_a–C(β)	1.97 (*t*, *J* = 11.5)	39.6			

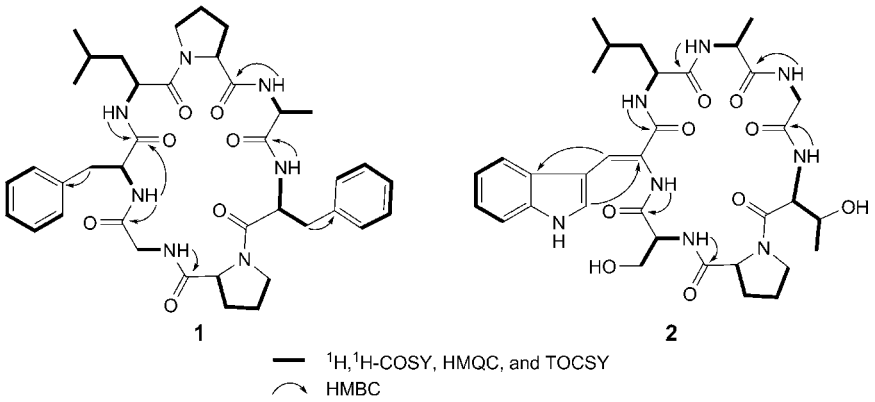

Fig. 2. *Selected 2D-NMR correlations for* **1** *and* **2**

combination with inspection of the HMBC spectrum. The CO C-atoms of Pro, Gly, Phe, Leu, Pro, Ala, and Phe were clearly assigned to δ(C) 172.7, 169.5, 172.6, 172.9, 172.4, 173.0, and 171.8, based on the observed HMBC correlations between CO groups and α- or β-H-atoms of the same amino acid residues, respectively.

The connectivity of amino acid residues was mainly established by NOESY cross-peaks: Phe^3-NH/HN-Gly^2, Leu^4-NH/HN-Phe^3, Pro^5-C(α)H/HC(α)-Leu^4, Phe^7-NH/HN-Ala^6, and Pro^1-C(δ)H$_2$/HC(α)-Phe^7 (*Fig. 3*). In conjunction with the HMBC correlations of Gly^2-NH/CO-Pro^1 and Ala^6-NH/CO-Pro^5 (*Fig. 2*), the backbone of **1** was thus determined as cyclo-(Pro^1-Gly^2-Phe^3-Leu^4-Pro^5-Ala^6-Phe^7). The strong NOE correlation between Phe^7-C(α)H and both δ_a, δ_b H-atoms of Pro^1 suggested the amide bond of Phe^7–Pro^1 as *trans*. However, the amide bond of Leu^4–Pro^5 was determined as *cis* because of the NOESY correlation Pro^5-C(α)H/HC(α)-Leu^4. These configurations were further confirmed by the ^{13}C chemical shifts of β-C-atom (δ(C) 29.3) and γ-C-atom (δ(C) 26.1) of Pro^1, and β-C-atom (δ(C) 32.9) and γ-C-atom (δ(C) 22.7) of Pro^5, respectively [12].

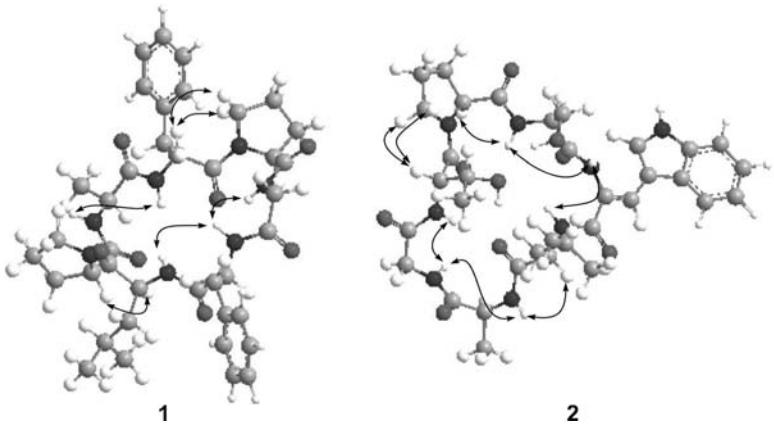

Fig. 3. *Key NOE correlations for* **1** *and* **2**

Compound **2** was isolated as colorless solid ($[\alpha]_D^{20} = -73.8$, $c = 0.155$, MeOH). The molecular formula was established as $C_{34}H_{46}N_8O_9$ by HR-ESI-MS (positive-ion mode; $[M+H]^+$ peak at m/z 711.3454; calc. 711.3461). Comparison of the molecular formula of **2** with that of tunicyclin C ($C_{34}H_{48}N_8O_9$) revealed that **2** had one unsaturation degree more than tunicyclin C. Furthermore, the ^{1}H- and ^{13}C-NMR spectra of **2** were also very similar to those of tunicyclin C (*Table 2*). Six amino acid residues of Pro, Ser, Leu, Ala, Gly, and Thr could be unambiguously deduced from their typical chemical shifts, and then confirmed by 2D-NMR experiments (^{1}H,^{1}H-COSY, HMQC and HMBC; *Fig. 2*) [10][11]. Furthermore, only six 'normal' α-amino acid C-atom resonances (δ(C) 62.0, 57.9, 57.6, 51.7, 51.1, and 43.9) were detected in ^{13}C-NMR spectrum of **2**. However, the presence of one 3-substituted indolyl moiety and one trisubstituted C=C bond in **2**, and the maximum UV absorbance band of **2** at 340 nm indicated that the cyclic peptide contains an α,β-dehydrotryptophan (Δ-Trp) residue [13]. The presence of the Δ-Trp residue was further confirmed by the HMBC correlations (*Fig. 2*) between the H–C(β) (δ(H) 8.60) of the trisubstituted olefin and the C-atom resonances at δ(C) 130.2 (C(2′) of indolyl group), and 165.9 (CO group of Δ-Trp residue). The CO C-atoms of Pro, Ser, Δ-Trp, Leu, Ala, Gly, and Thr were also assigned to δ(C) 172.5, 171.0, 165.9, 175.9,

Table 2. *^{1}H- and ^{13}C-NMR Data of* **2** (at 500 and 125 MHz, resp., in C_5D_5N, J in Hz, δ in ppm)

Residue	δ(H)	δ(C)	Residue	δ(H)	δ(C)
Pro[1]			H–C(7′)	7.57 (*d*, $J = 7.8$)	112.2
CO		172.5	C(7a′)		136.5
H–C(α)	4.61 – 4.66 (*m*)	62.0	*Leu*[4]		
H$_a$–C(β)	2.24 – 2.32 (*m*)	29.3	CO		175.9
H$_b$–C(β)	1.97 – 2.05 (*m*)		NH	8.19 (*d*, $J = 9.3$)	
H$_a$–C(γ)	1.74 – 1.80 (*m*)	25.2	H–C(α)	5.30 (*dd*, $J = 9.3.\ 3.5$)	51.7
H$_b$–C(γ)	1.48 – 1.54 (*m*)		H$_a$–C(β)	1.78 – 1.83 (*m*)	41.4
H$_a$–C(δ)	3.67 – 3.74 (*m*)	48.3	H$_b$–C(β)	1.63 – 1.70 (*m*)	
H$_b$–C(δ)	3.57 – 3.64 (*m*)		H–C(γ)	1.80 – 1.87 (*m*)	24.6
Ser[2]			Me(δ_a)	0.76 (*d*, $J = 6.2$)	22.6
CO		171.0	Me(δ_b)	0.66 (*d*, $J = 6.2$)	21.7
NH	8.28 (*d*, $J = 6.8$)		*Ala*[5]		
H–C(α)	4.96 – 5.00 (*m*)	57.9	CO		172.3
H$_a$–C(β)	4.58 – 4.62 (*m*)	62.5	NH	11.09 (s)	
H$_b$–C(β)	4.21 (*d*, $J = 10.8$)		H–C(α)	4.29 – 4.35 (*m*)	51.1
Δ^Z-*Trp*[3]			Me(β)	1.73 (*d*, $J = 6.9$)	15.9
CO		165.9	*Gly*[6]		
NH	9.46 (s)		CO		170.3
H–C(α)		121.6	NH	9.99 (*dd*, $J = 6.3, 5.8$)	
H–C(β)	8.60 (s)	128.0	H$_a$–C(α)	4.60 (*dd*, $J = 17.1, 6.3$)	43.9
NH(1′)	13.1 (s)		H$_b$–C(α)	3.76 (*dd*, $J = 17.1, 5.8$)	
H–C(2′)	8.97 (*d*, $J = 2.4$)	130.2	*Thr*[7]		
C(3′)		109.9	CO		172.2
C(3a′)		128.7	NH	8.34 (*d*, $J = 9.1$)	
H–C(4′)	7.71 (*d*, $J = 7.8$)	118.2	H–C(α)	5.41 (*dd*, $J = 9.1, 2.8$)	57.6
H–C(5′)	7.15 (*t*, $J = 7.8$)	120.4	H–C(β)	4.65 – 4.72 (*m*)	67.7
H–C(6′)	7.23 (*t*, $J = 7.8$)	122.2	Me(γ)	1.63 (*d*, $J = 6.4$)	19.4

172.3, 170.3 and 172.2 based on the observed HMBCs between CO groups and α- or β-H-atoms of the same amino acid residue, respectively.

The amino acid sequence (cyclo-(Pro^1-Ser^2-Δ-Trp^3-Leu^4-Ala^5-Gly^6-Thr^7)) of **2** was mainly established by the following NOESY cross-peaks: Ser^2-NH/HC(α)-Pro^1, Δ-Trp^3-NH/HN-Ser^2, Leu^4-NH/HN-Δ-Trp^3, Ala^5-NH/HC(α)-Leu^4, Gly^6-NH/HN-Ala^5, Thr^7-NH/HN-Gly^6, and Pro^1-C(δ)H$_2$/HC(α)-Thr^7 (*Fig. 3*). The configuration of Δ-Trp residue was established as (*Z*) on the basis of the chemical shift of β-H-atom (δ(H) 8.60) of Δ-Trp [8]. This result was supported by the ROESY correlation between Δ-Trp^3-C(2′)H (δ(H) 8.97) and Ser^2-C(α)H (δ(H) 4.98). The strong NOE correlation between Thr^7-C(α)H and both δ_a- and δ_b-H-atoms of Pro^1 suggested the amide bond of Thr^7–Pro^1 as *trans*. The result was further confirmed by the ^{13}C chemical shifts of β- and γ-C-atoms of Pro^1 at δ(C) 29.3 and 25.2 ppm, respectively, in agreement with those of *trans*–Pro [11].

Experimental Part

General. Column chromatography (CC): macroporous resin (*Diaion HP-20*), C_{18} reversed-phase (RP) silica gel (*ODS*, 50 μm; *YMC*, Japan), silica gel (SiO_2; 200–300 mesh; *Huiyou Silical Gel Development Co. Ltd.*, Yantai, P. R. China), silica gel *H* (10–40 μm, Qingdao, P. R. China), and *Sephadex LH-20* (40–70 μm; *GE Heathcare Bio-Sciences AB*, USA). TLC: *HSGF254* silica gel plates (10–40 μm, *Huiyou Silica Gel Development Co., Ltd.*, Yantai, P. R. China). Optical rotations: *Perkin-Elmer 341* polarimeter. UV Spectra: *UV-2550* UV/VIS spectrophotometer (*Shimadzu*, Japan). IR Spectra: *FT-IR 6700* spectrometer (*Nicolet*, USA), KBr pellets. 1D- and 2D-NMR spectra: *Bruker Avance 500* NMR spectrometer in C_5D_5N, δ in ppm, *J* in Hz. ESI-MS: *LC/MSD Trap XCT* (*Agilent*, USA). HR-ESI-MS: *Accurate-Mass-Q-TOF LC/MS 6520* (*Agilent*, USA).

Plant Material. The roots of *Psammosilene tunicoides* (40 kg) were collected in Lijiang, Yunnan Province, P. R. China, in 2006. The identification was performed by Prof. *Li-Shan Xie*, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (herbarium No. 2006071015) is deposited with the School of Pharmacy, Second Military Medical University, P. R. China.

Extraction and Isolation. The air-dried powdered material was refluxed with 80% EtOH (10 h). The residue obtained by evaporating the solvent was partitioned between H_2O and $CHCl_3$, and then the $CHCl_3$-soluble extract (285 g) was subjected to CC (SiO_2 100–200 mesh); column successively eluted with petroleum ether (PE)/AcOEt (1, 5, 10, 20, 30, and 40%; then, with 10% MeOH/$CHCl_3$) to yield nine fractions (*F1–F9*). The H_2O-soluble extract was submitted to CC (*Diaion HP-20*; successively eluted with H_2O, 70% EtOH, and acetone) to yield two fractions: acetone fraction (35.5 g) and 70% EtOH fraction (7.5 kg). The acetone fraction was combined with the fraction *F9* to afford *Fr. M* which was subjected to CC (*MCI* gel; successively eluted with H_2O, 70% MeOH, and MeOH) to yield two fractions, *M-1* and *M-2*. *Fr. M-1* was subjected to CC (SiO_2; MeOH/$CHCl_3$ 5, 10, 15, 20, and 30%) to give four subfractions, *M-1-1* to *M-1-4*). *Fr. M-1-2* was further purified by repeated RP silica gel (*ODS*) and *Sephadex LH-20* CC to afford compound **1** (159 mg). Parts of the 70% EtOH fraction (1 kg) was subjected to CC (RP silica gel (*ODS*); eluted successively with a gradient of EtOH/H_2O) to afford six fractions, *S1* to *S6*. *Fr. S1* was further separated into three fractions, *S1-1*, *S1-2*, and *S1-3* by CC on RP silica gel (*ODS*). *Fr. S1-2* was subjected to CC (SiO_2; gradient $CHCl_3$/MeOH) to afford five fractions (*S1-2-1* to *S1-2-5*). *Fr. S1-2-3* was further purified by repeated RP silica gel (*ODS*) and *Sephadex LH-20* CC to afford compound **2** (4 mg).

Tunicyclin F (= *cyclo-(Pro^1-Gly^2-Phe^3-Leu^4-Pro^5-Ala^6-Phe^7)* = *cyclo*(L-*Alanyl*-L-*phenylalanyl*-L-*prolylglycyl*-L-*phenylalanyl*-L-*leucylprolyl)*; **1**): Colorless solid. $[\alpha]_D^{20} = -74.6$ ($c = 0.135$, MeOH). UV (MeOH): 202. IR (KBr): 3311, 2959, 2956, 1633, 1520, 1452, 1241, 748, 700. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS: 728 ($[M-H]^-$), 730 ($[M+H]^+$). HR-ESI-MS (pos.): 730.3927 ($[M+H]^+$, $C_{39}H_{51}N_7O_7^+$; calc. for 730.3923).

Tunicyclin G (= *cyclo-(Pro*^{1}*-Ser*^{2}*-Δ-Trp*^{3}*-Leu*^{4}*-Ala*^{5}*-Gly*^{6}*-Thr*^{7}*)* = *(3*S*,6*Z*,9*S*,12*S*,18*S*,23a*S*)-18-(1-Hydroxyethyl)-3-(hydroxymethyl)-6-[(1*H*-indol-3-yl)methylidene]-12-methyl-9-(2-methylpropyl)hexadecahydro-1*H*-pyrrolo[1,2-*a*][1,4,7,10,13,16,19]heptaazacyclohenicosine-1,4,7,10,13,16,19-heptone*; **2**): Colorless solid. $[\alpha]_D^{20} = -73.8$ ($c = 0.155$, MeOH). UV (MeOH): 340. IR (KBr): 3419, 2929, 1643, 1516, 1458, 1246, 1074, 748, 561. ^{1}H- and ^{13}C-NMR: see *Table 2*. ESI-MS: 709 ($[M-H]^-$), 733 ($[M+Na]^+$). HR-ESI-MS (pos.): 711.3454 ($[M+H]^+$, $C_{34}H_{47}N_8O_9^+$; calc. for 711.3461).

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