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Tunicyclin A, the First Plant Tricyclic Ring Cycloheptapeptide from *Psammosilene tunicoides*

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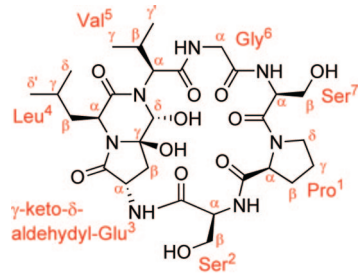
ABSTRACT



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A novel cycloheptapeptide, tunicyclin A, with a unique tricyclic ring cyclopeptide skeleton, was isolated from *Psammosilene tunicoides*. Its structure was elucidated by extensive NMR and MS analysis. Biogenetically, tunicyclin A might be derived from *cyclo-(Pro[1]-Ser[2]-(γ-keto-δ-aldehedyl-Glu[3])-Leu[4]-Val[5]-Gly[6]-Ser[7])* via two steps of nucleophilic addition.

Psammosilene tunicoides W. C. Wu et. C. Y. Wu, a monotype genus plant belonging to the Caryophyllaceae family, is one of the important ingredients of a famous Chinese traditional medicine formulation "Yunnan Baiyao". Also, this plant is commonly used as an anodyne and haemastatic agent in southwest China.[1,2] Previous phytochemical studies on this plant have afforded triterpenoid saponins and cyclopeptides.[3,4] As part of our investigation on structurally and pharmacologically interesting secondary metabolites from Chinese medicinal plants, an unprecedented

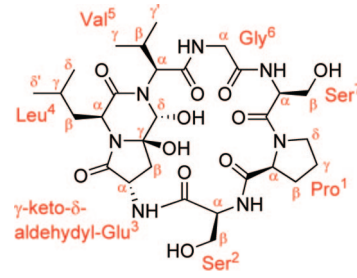

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Figure 1. Chemical structure of **1**.

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tricyclic ring cycloheptapeptide, tunicyclin A (**1**) (Figure 1), was isolated from the titled plant. Herein we describe the isolation and structural elucidation of **1**.

Tunicyclin A (**1**) was isolated as a white solid.[5] Its molecular formula was established as $C_{29}H_{45}N_7O_{11}$ by positive HR-Q-TOF-MS (*m/z* [M + Na]$^+$ 690.30764; cacld 690.30747). The ^{1}H NMR spectrum of **1** (Table 1) displayed

Table 1. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) Data of **1** in C_5D_5N[a]

	δ_H	δ_C		δ_H	δ_C
Pro[1]			Leu[4]		
CO		171.8	CO		169.2
α	4.84 (dd, 8.7, 5.7)	61.7	α	4.98 (dd, 8.4, 5.4)	52.6
β_a	2.06 (m)	29.4	β	2.20 (2H, ddd, 8.4, 5.4, 2.4)	45.2
β_b	2.00 (m)		γ	2.00 (m)	25.0
γ_a	1.48 (m)	24.8	δ	0.76 (3H, d, 6.6)	23.2
γ_b	1.65 (m)		δ'	1.08 (3H, d, 6.6)	21.7
δ_a	3.40 (dt, 9.6, 7.2)	48.2	Val[5]		
δ_b	3.91 (dt, 9.6, 7.2)		CO		172.4
Ser[2]			α	5.53 (d, 10.8)	59.8
CO		171.4	β	2.66 (m)	26.3
NH	8.29 (d, 7.8)		γ	1.16 (3H, d, 6.0)	21.0
α	5.08 (ddd, 7.8, 5.4, 3.0)	57.4	γ'	1.20 (3H, d, 6.6)	18.1
β_a	4.49 (dd, 10.8, 5.4)	62.4	Gly[6]		
β_b	4.22 (dd, 10.8, 3.0)		CO		169.6
Glu[3]			NH	9.19 (dd, 6.6, 5.4)	
CO		170.6	α_a	4.82 (dd, 17.1, 6.3)	44.2
NH	8.52 (d, 8.4)		α_b	3.95 (dd, 17.1, 5.4)	
α	5.46 (ddd, 9.6, 8.4,6.0)	49.6	Ser[7]		
β_a	2.97 (dd, 15.0, 9.6)	40.0	CO		171.7
β_b	2.94 (dd, 15.0, 6.0)		NH	8.42 (d, 9.6)	
γ		87.6	α	5.56 (dt, 9.6, 6.0)	51.3
γ-OH	8.52 (s)		β_a	4.04 (t, 9.6)	64.0
δ	5.43 (d, 7.2)	80.4	β_b	3.95 (dd, 9.6, 6.0)	
δ-OH	8.60 (d, 7.2)				

[a] All proton signals integrate to 1H, unless otherwise indicated.

six amide NH or hydroxyl proton signals resonating at δ_H 8.29 (d), 8.42 (d), 8.52 (d), 8.52 (s), 8.60 (d), and 9.19 (dd). The ^{13}C NMR spectrum (Table 1) exhibited seven amide carbonyl resonances at δ_C 169.2, 169.6, 170.6, 171.4, 171.7, 171.8, and 172.4, along with seven α-amino acid carbons resonating at δ_C 61.7, 59.8, 57.4, 52.6, 51.3, 49.6, and 44.2. On the basis of the above data, together with its negative reaction to ninhydrin, **1** was inferred to be a typical cycloheptapeptide. Also, the 1D NMR spectra indicated several side chain groups, including four methyls (due to two isopropyl groups), four methylenes, one CH_2N group, two CH_2OH groups, and two methines. However, one oxygenated sp^3 quaternary carbon (δ_C 87.6) and one oxygenated sp^3 methine (δ_C 80.4) remained unknown.

Completed assignment for protons and carbons of **1** was addressed by 2D NMR experiments, including COSY, TOCSY, HMQC, and HMBC. From ^{1}H−^{1}H COSY and TOCSY experiments, seven spin coupling systems of Pro, Ser, Glu (absence of the γ methylene protons), Leu (absence of the NH proton), Val (absence of the NH proton), Gly, and Ser were observed, respectively (Figure 2).[6,7] Furthermore, the carbonyl carbons of Pro, Ser, Glu, Leu, Val, Gly,

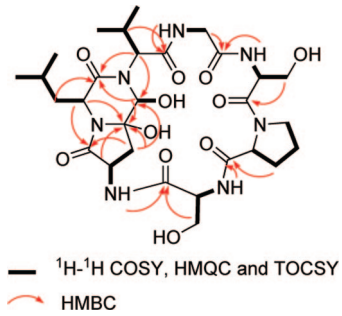

Figure 2. Selected 2D NMR correlations for **1**.

and Ser were undoubtedly assigned to δ_C 171.8, 171.4, 170.6, 169.2, 172.4, 169.6, and 171.7 based on correlations between carbonyl carbons and α or β protons of the same amino acid residues in HMBC experiment, respectively. In addition, the quaternary carbon at δ_C 87.6 was determined as the γ carbon of the Glu residue by HMBC correlations from Glu-αH, β_aH, and β_bH to δ_C 87.6, while the methine at δ_C 80.4 was assigned to δ carbon of the Glu residue by HMBC correlations from Glu-β_aH and β_bH to δ_C 80.4.

The peptide sequence and connectivity of amino acid residues were established by HMBC crosspeaks: Ser^2-NH/CO-Pro^1, Glu^3-NH/CO-Ser^2, Leu^4-αH/CO-Glu^3, Val^5-αH/CO-Leu^4, Gly^6-NH/CO-Val^5, Ser^7-NH/CO-Gly^6 (Figure 2). In conjunction with ROESY correlations of Ser^7-αH with δ_a and δ_b protons of Pro^1, the backbone of **1** was thus determined as *cyclo-(Pro^1-Ser^2-Glu^3-Leu^4-Val^5-Gly^6-Ser^7)* (Figure 2 and 3). Since **1** had 11 degrees of unsaturation, while seven amino acid residues and the macrocycle simply accounted for nine degrees of unsaturation, **1** should bear another two rings, and the γ and δ carbons of the Glu residue should participate in the cyclization. Further, HMBC correlation of Leu-αH with γ carbon of Glu indicated that γ carbon of Glu was connected to the amino group of the Leu residue. Additionally, HMBC correlation of Val-αH with δ carbon of Glu revealed that δ carbon of Glu was linked to the amino group of Val residue. The connectivity was also confirmed by HMBC correlation of Glu^3-δH to carbonyl carbon of Leu^4 residue. On the basis of the above evidence, the planar structure of tunicyclin A (**1**) was constructed.

The absolute configurations of Pro^1, Ser^2, Leu^4, Val^5, and Ser^7 were identified as L (*S*) on the basis of HPLC-ESI-MS analysis of the retention times and *m/z* values of the chiral derivatives of the amino acid residues in acid hydrolysate of **1** (see detailed information in the Supporting Information).[8] Since Val^5-αH was in *trans* configuration to Val^5-βH based on the coupling constant of Val^5-αH/Val^5-βH (*J*

(5) Tunicyclin A (**1**): $[\alpha]_D^{20}$ −47.0 (*c* 0.10, MeOH); IR (KBr)υ_{max} 3338, 2959, 2874, 1653, 1541, 1457, 1056, 706 cm^{-1}. UV (MeOH) λ_{max} 211, 256 nm. Positive ESI-MS *m/z*: 690 [M + Na]$^+$. Negative ESI-MS *m/z*: 666 [M − H]$^-$. Positive HR-Q-TOF-MS *m/z*: 690.30764 [M + Na]$^+$, cacld. for $C_{29}H_{45}N_7O_{11}Na$, 690.30747.

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= 10.8 Hz), strong ROESY correlation between Val[5]-βH and Glu[3]-δH suggested that the configuration of the δ carbon of Glu[3] was *R*. Furthermore, ROESY correlation between γ-OH of Glu[3] and Leu[4]-βH implied that the configuration of γ carbon of Glu[3] was *R*. Strong NOE correlation between Ser[7]-αH and both δ_a, δ_b protons of Pro[1] suggested that the amide bond of Ser[7]-Pro[1] was *trans*. Although there is no direct evidence, considering that all naturally occurring amino acids from high plant are an L configuration, together with ROESY correlation of γ-OH of Glu[3] with Glu[3]-αH, the absoulte configuration of Glu[3] still could be assigned as L (*S*). Consequently, the stereoconfiguration of **1** was determined (shown in Figure 3).

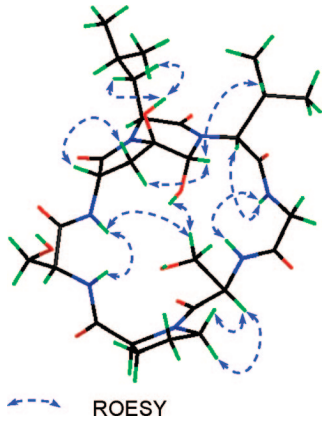
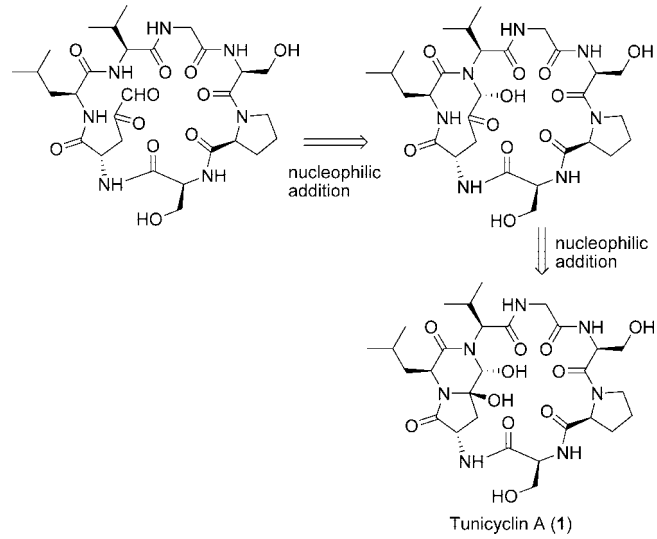


Figure 3. 3D drawing of **1** with selected diagnostic NOEs. Configurations at α, γ, and δ positions of proposed Glu.

Tunicyclin A (**1**) contains an unusual amino acid residue, γ-keto-δ-aldehydyl-Glu. The γ and δ carbonyl carbons of the γ-keto-δ-aldehydyl-Glu residue participate in the cyclization with the NH of Leu[4] and Val[5], respectively, and form a unique cycloheptapeptide backbone with a tricyclic ring system. To the best of our knowledge, tunicyclin A is the first plant tricyclic cyclopeptide and represents a new type of cyclopeptide. Biogenetically, this tricyclic cycloheptapeptide might originate from *cyclo-(Pro[1]-Ser[2]-(γ*-keto-δ-aldehydyl-*Glu[3])-Leu[4]-Val[5]-Gly[6]-Ser[7])* via two steps of nucleophilic addition (Scheme 1).

Scheme 1. Proposed Biogenetic Pathway of **1**



Tunicyclin A (**1**) was evaluated in vitro for cytotoxicity against four human cancer cell lines, A549, LOVO, HL-60, and L-929, using MTT assay with DOX (doxorubicin) as a positive control,[9] but showed no inhibitory activity against four tested cell lines.

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Supporting Information Available: Experimental section and 1D and 2D NMR spectra of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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