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

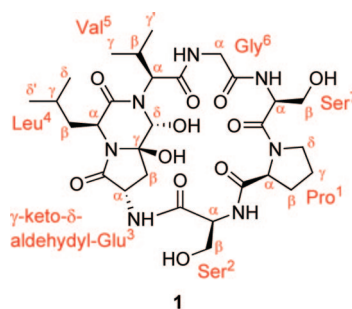
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ABSTRACT



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*-(Pro¹-Ser²-(γ -keto- δ -aldehydyl-Glu³)-Leu⁴-Val⁵-Gly⁶-Ser⁷) 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

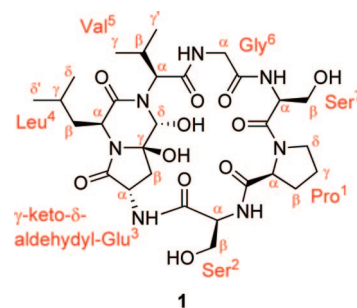


Figure 1. Chemical structure of 1.

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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 C₂₉H₄₅N₇O₁₁ by positive HR-Q-TOF-MS (*m/z* [M + Na]⁺ 690.30764; *ca*cd 690.30747). The ¹H NMR spectrum of **1** (Table 1) displayed

Table 1. ¹H (600 MHz) and ¹³C NMR (150 MHz) Data of **1** in C₅D₅N^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, 9.6)	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 ¹³C 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 CH₂N group, two CH₂OH groups, and two methines. However, one oxygenated sp³ quaternary carbon (δ_C 87.6) and one oxygenated sp³ 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 ¹H–¹H 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,

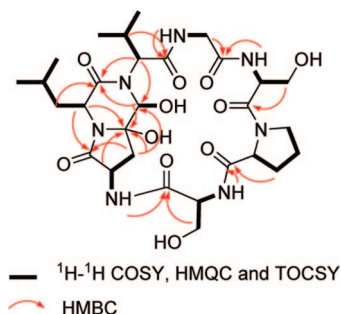


Figure 2. Selected 2D NMR correlations for **1**.

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: Ser²-NH/CO-Pro¹, Glu³-NH/CO-Ser², Leu⁴-αH/CO-Glu³, Val⁵-αH/CO-Leu⁴, Gly⁶-NH/CO-Val⁵, Ser⁷-NH/CO-Gly⁶ (Figure 2). In conjunction with ROESY correlations of Ser⁷-αH with δ_a and δ_b protons of Pro¹, the backbone of **1** was thus determined as *cyclo*-(Pro¹-Ser²-Glu³-Leu⁴-Val⁵-Gly⁶-Ser⁷) (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 Glu³-δH to carbonyl carbon of Leu⁴ residue. On the basis of the above evidence, the planar structure of tunicyclin A (**1**) was constructed.

The absolute configurations of Pro¹, Ser², Leu⁴, Val⁵, and Ser⁷ 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 Val⁵-αH was in *trans* configuration to Val⁵-βH based on the coupling constant of Val⁵-αH/Val⁵-βH (*J*

(5) Tunicyclin A (**1**): [α]_D²⁰ –47.0 (c 0.10, MeOH); IR (KBr) ν_{\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]⁺, *ca*cd. for C₂₉H₄₅N₇O₁₁Na, 690.30747.

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= 10.8 Hz), strong ROESY correlation between Val⁵-βH and Glu³-δH suggested that the configuration of the δ carbon of Glu³ was *R*. Furthermore, ROESY correlation between γ-OH of Glu³ and Leu⁴-βH implied that the configuration of γ carbon of Glu³ was *R*. Strong NOE correlation between Ser⁷-αH and both δ_a, δ_b protons of Pro¹ suggested that the amide bond of Ser⁷-Pro¹ 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 Glu³ with Glu³-αH, the absolute configuration of Glu³ still could be assigned as L (*S*). Consequently, the stereoconfiguration of **1** was determined (shown in Figure 3).

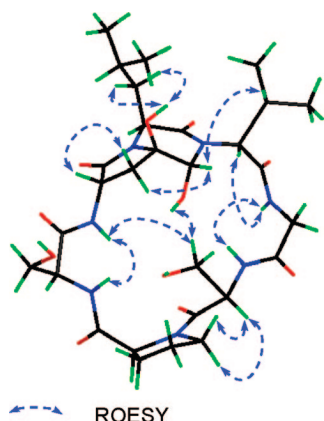
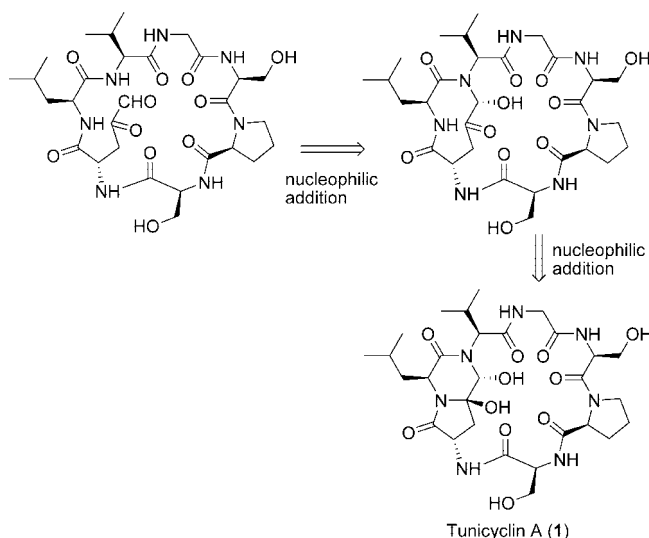


Figure 3. 3D drawing of **1** with selected diagnostic NOEs. Configurations at α, γ, and δ positions of proposed Glu.

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 Leu⁴ and Val⁵, 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 cycloheptapeptide and represents a new type of cycloheptapeptide. Biogenetically, this tricyclic cycloheptapeptide might originate from *cyclo*-(Pro¹-Ser²-(γ-keto-δ-aldehydyl-Glu³)-Leu⁴-Val⁵-Gly⁶-Ser⁷) 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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