SHORT REPORT

ACG publications

Rec. Nat. Prod. 17:1 (2023) 179-183

records of natural products

Psammosilenin C, a New Cyclic Peptide from *Psammosilene tunicoides*

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(Received March 27, 2022; Revised May 31, 2022; Accepted June 04, 2022)

Abstract: Phytochemical investigation of *Psammosilene tunicoides* led to isolation of a new cyclicpeptide, psammosilenin C (**1**) and three known compounds, 1-O-(*β*-D-glucopyranosyl)-(2*S*,3*R*,4*E*,8*E*)-2-[(2*R*)-2-hydroxypentadecanoylamino]-4,8-octadecadiene-1,3-diol (**2**), dihydroferulic acid (**3**) and vanillylacetone (**4**). Their structures were elucidated by comprehensive spectroscopic methods, including 1D and 2D NMR spectroscopic, and HR-ESI-MS spectrometric data. Compounds **1**, **2** and **4** showed inhibitory activities on lipopolysaccharide (LPS)-induced NO release in RAW 264.7 cells.

Keywords: *Psammosilene tunicoides*; cyclic peptide; anti-inflammatory activity. © 2022 ACG Publications. All rights reserved.

1. Plant Source

The roots of *Psammosilene tunicoides* W. C. Wu & C. Y. Wu were collected in 2019 from Yuxi of Yunnan province in China and authenticated by Prof. Qingsong Yang, the School of Ethnic Medicine, Yunnan Minzu University, China. A voucher specimen (No.201910JTS) has been deposited at the herbarium of the above Department.

2. Previous Studies

Psammosilene tunicoides, the only species of *Psammosilene* genus belonging to Cayrophyllaeeae, is a well-known ethnic medicine and successfully used as an anodyne and hemostatic agent[1]. Pharmacological studies emphasized that the triterpenoid saponins were main active constituents of *P. tunicoides*, which could be used for anti-inflammation, analgesic, and the regulation of immune function [2-4]. Moreover, the cyclic peptides were considered as one of characteristic compositions in *P. tunicoides*. The discovery of the first cyclic peptides in this plant was

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The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products January-February 2023 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.336.2203.2399
Available online: June 21, 2022

two cyclic octapeptides, psammosilenins A and B, isolated by Jun Zhou research team [5]. Seven cyclic dipeptides, including cyclo (-Pro-Pro-), cyclo (-Pro-Val-), cyclo (-Pro-Ala-), cyclo (-Ala-Ala-), cyclo (-Ala-Ile-), cyclo (-Ala-Leu-), and cyclo (-Ala-Val-), were found from the roots of *P. tunicoides* [6,7]. Series of cyclic heptapeptides, tunicyclins A-C, E-L, K, and a cyclic octapeptide, tunicyclin D, was isolated from *P. tunicoides*, a few of which possessed unique tricyclic ring cyclopeptide skeleton and appreciable bioactivities, such as anti-acetylcholinesterase and antifungal activities [8-13].

In our work, four compounds were isolated from *P. tunicoides*, including a new cyclic peptide (**1**), a cerebroside (**2**) and two phenylpropanoic acid derivatives (**3**, **4**). Notably, compound **1** was the first cyclic nonapeptide obtained from this plant, indicating that the cyclopeptides were very abundant and diverse in *P. tunicoides*. Up to now, cyclic dipeptides, cyclic heptapeptides, cyclic octapeptides, and the cyclic nonapeptide were mentioned. Cerebroside **2** was previously isolated from *Allium sativum* [14], *Arisaema flavum* [15], and *Arisaema amurense* [16], suggested that cerebrosides were one of main constituents in the plant of genus *Arisaema*. The cerebrosides in *P. tunicoides* have been less concerned, and soya-cerebroside was only one reported so far. Compounds **3** and **4** were the group of phenylpropanoic constituents widely distributed in nature plants, e.g. *Nicandra physaloides* [17], *Micromelum minutum* [18], *Malva silvestris* [19], etc. Our present research enriches the chemical diversity and affords fundament for the further chemotaxonomic studies of *P. tunicoides*.

3. Present Study

The dried roots of *P. tunicoides* were powdered and macerated with 95% EtOH (15.0 L × 3) at room temperature. The organic solvent was removed at reduced pressure to yield a crude extract, which was suspended in H_2O and continuously extracted by EtOAc. The EtOAc fraction was subjected to silica gel CC eluting with gradients of $CHCl_2$: MeOH (100:1-0:100, v/v) to yield four fractions (Frs.I~IV). Fr.I fraction was chromatographed by Sephadex LH-20 column (100% MeOH) and then was purified by silica gel CC eluting with $CHCl_2$: EtOAc (9:1, v/v) to give compound **4** (10.0 mg). Fr.II fraction was chromatographed by Sephadex LH-20 column (100% MeOH) to yield two subfractions, Frs.II-1 and II-2. Fr.II-1 was purified by semi-preparative HPLC (MeOH:H_2O, 73:27, v/v) to give compound **1** (8.0 mg). Fr.II-2 was subjected to silica gel CC eluting with $CHCl_2$: MeOH (100:1, v/v) to afford compound **3** (6.5 mg). Fr.III fraction was separated by Sephadex LH-20 CC and eluted with 100% MeOH to obtain compound **2** (30.1 mg).

*Psammosilenin C (**1**)*: colorless crystal, mp. 247-249 °C; $[\alpha]_D^{26.7}$-250 (*c* 0.02, MeOH); IR (KBr) ν_{max}: 3400, 2964, 2877, 2361, 2027, 1634, 1517, 1450, 1350, 1240, 1073, and 541cm^{-1}; ^{1}H- and ^{13}C-NMR spectral data, see Table 1; HR-ESI-MS *m/z*: 942.5296 $[M+H]^+$ (calcd. for $C_{46}H_{72}N_9O_{12}$, 942.5295).

Anti-inflammatory activity tests: RAW264.7 cells were obtained from Kunming Institute of Zoology, Chinese Academy of Sciences (Yunnan, China). The cell viability was detected by the MTT colorimetric assay, after 24h of incubation with test compounds. For the NO assay, the RAW264.7 cells (1×10^5 cells/well) were incubated in 96-well plates with various concentrations of test samples and co-incubated with LPS (1 μg/mL) for 24 h. The Griess reagent was mixed with culture medium and incubated at room temperature for 15 min, after which the absorbance at 540 nm was measured in a microplate reader.

The ethanol extract from the roots of *P. tunicoides* was subjected to conventional purification procedures and affording a new cyclic peptide, psammosilenin C (**1**) (Figure 1), a ceramide, 1-O-(*β*-D-glucopyranosyl)-(2*S*,3*R*,4*E*,8*E*)-2-[(2*R*)-2-hydroxypentadecanoylamino]-4,8-octadecadiene-1,3-diol (**2**), and two phenolic compounds, dihydroferulic acid (**3**) and vanillylacetone (**4**).

Compound **1**, psammosilenin C, was isolated as a colorless crystal with a molecular formula of $C_{46}H_{71}N_9O_{12}$, established by positive HR-ESI-MS (*m/z* 942.5296 $[M+H]^+$; calcd. 942.5295). The ^{13}C-NMR spectral data (Table 1) exhibited typical carbon resonance of nonapeptide, i.e., nine amide carbonyls (δ_C 174.9, 174.7, 174.3, 173.9, 173.6, 173.2, 172.9, 172.8, and 171.1) along to nine normal α-amino acid carbons (δ_C 63.7, 62.3, 60.7, 60.5, 59.4, 56.1, 53.4, 52.8, and 42.2). The extensive analysis of HSQC, ^{1}H-^{1}H COSY and TOCSY correlations (Supporting Information, Figure S5,S12 and S13) suggested that the cyclicpeptide consist of tyrosine (Tyr), threonine (Thr), isoleucine (Ile, ×2), leucine (Leu), serine (Ser), proline (Pro, ×2), and glycine (Gly). By further inspection of ^{1}H-NMR,

^{13}C-NMR, HSQC spectra, and HMBC correlations between carbonyls and α- or β- protons of the same amino acid residues, the assignment of each α proton and the amide carbonyl carbon were determined (Table 1).

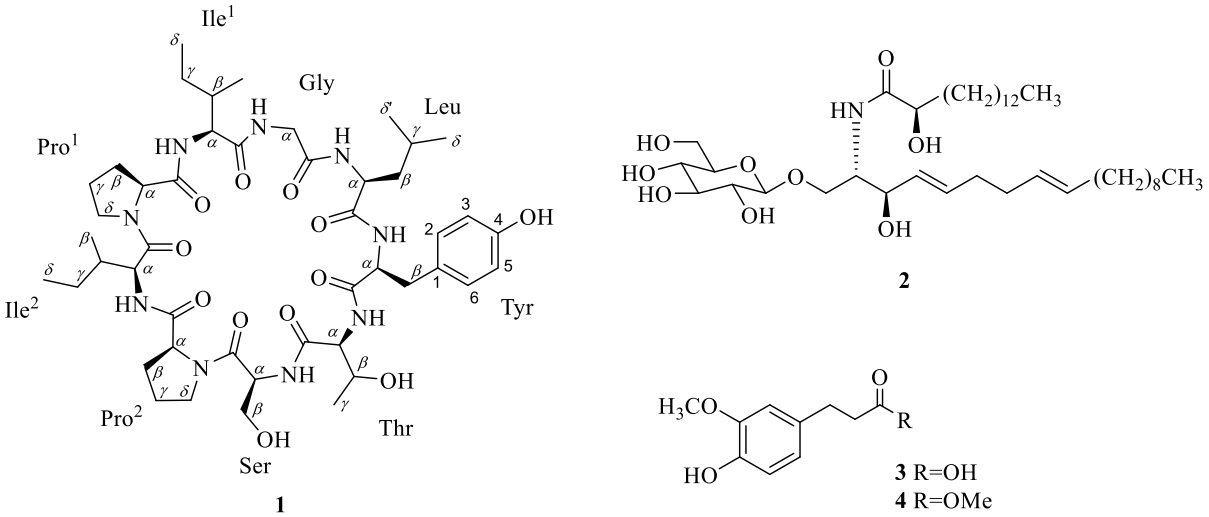

Figure 1. Chemistry structure of compounds **1**-**4**.

Table 1. ^{1}H- and ^{13}C-NMR data of compound **1** in CD_3OD ([a]400 MHz, [b]100 MHz)

Position		δ_H (*J* in Hz)[a]	δ_C, mult.[b]	Position		δ_H (*J* in Hz)[a]	δ_C, mult.[b]
Pro^1	CO		173.6, C		1		127.6, C
	α	3.61 (d, *J* = 7.8 Hz)	62.3, CH		2/6	7.10 (d, *J* = 8.5 Hz)	132.0, CH
	β	1.06, m; 2.03, m	31.5, CH_2		3/5	6.69 (d, *J* = 8.5 Hz)	116.6, CH
	γ	1.43, m; 1.72, m	22.8, CH_2		4		158.0, C
	δ	3.37, m, 2H	47.6, CH_2	Thr	CO		172.9, C
Ile^1	CO		174.3, C		*α*	4.58, m	59.4, CH
	α	4.03 (d, *J* = 10.0 Hz)	60.5, CH		*β*	4.50 (dd, *J* = 6.5, 1.8 Hz)	68.4, CH
	β	2.14, m	35.2, CH		*γ*	1.10 (d, *J* = 6.5 Hz)	20.1, CH_3
	γ	1.53, m; 1.24, m	26.4, CH_2	Ser	CO		172.8, C
	δ	0.86 (t, *J* = 7.4 Hz)	10.4, CH_3		*α*	4.89, m	53.4, CH
	β-Me	0.91 (d, *J* = 6.4 Hz)	16.0, CH_3		*β*	4.06, m; 3.95, m	63.3, CH_2
Gly	CO		171.1, C	Pro^2	CO		174.7, C
	α	3.95, m	42.2, CH_2		*α*	4.35 (t, *J* = 7.7 Hz)	63.7, CH
Leu	CO		174.9, C		*β*	2.39, m; 1.94, m	30.9, CH_2
	α	4.47 (dd, *J* = 10.8, 4.1 Hz)	52.8, CH		*γ*	2.07, m, 2H	26.2, CH_2
	β	1.75, m; 1.58, m	40.8, CH_2		*δ*	3.94, m, 2H	49.2, CH_2
	γ	1.68, m	25.9, CH	Ile^2	CO		173.9, C
	δ	0.94 (d, *J* = 6.4 Hz)	21.6, CH_3		*α*	4.23 (d, *J* = 7.3 Hz)	60.7, CH
	δ′	0.97 (d, *J* = 6.4 Hz)	23.6, CH_3		*β*	1.95, m	37.2, CH
Tyr	CO		173.2, C		*γ*	1.53, m; 1.24, m	26.8, CH_2
	α	4.67 (dd, *J* = 11.5, 4.6 Hz)	56.1, CH		*δ*	0.92 (t, *J* = 7.8 Hz)	11.2, CH_3
	β	3.26 (dd, *J* = 12.5, 4.6 Hz) 2.84 (t, *J* = 12.5 Hz)	37.9, CH_2		*β*-Me	0.98 (d, *J* = 6.8 Hz)	16.3, CH_3

The plane structure of compound **1** were indicated by the HMBC correlations of Ser-H*α*/Thr-CO, Thr-H*α*/Tyr-CO, Leu-H*α*/Gly-CO, Gly-H*α*/Ile^1-CO, and Ile^1-H*α*/Pro^1-CO, and NOESY correlations of Pro^1-H*β*/Ile^1-H*δ*, Pro^1-H*β*/Ile^2-*β*-Me, Pro^2-H*β*/Ile^2-H*δ*, Pro^2-H*δ*/Ser-H*β*, Thr-H-2/Leu-H*α*, and Thr-H-6/Thr-H*γ* (Figure 2). By combine analyses of HR-ESI-MS^2 fragmentation data, the sequence of the nine amino acid units was confirmed (Figure 2). The observed parent ion *m/z* 942 $[M+H]^+$ or 924 $[M+H-H_2O]^+$ implied that the molecule was fragmented and opened between Pro^1 and Ile^2 residues. Fragment ions as shown in Figure 2 generated by the continuous fragmentation process

were in agreement with the sequence determined by 2D NMR analysis. The seven amino acid residues (Tyr, Thr, Ile, Leu, Ser, Pro, and Gly) present in some cyclicpeptide from the same plant were identified as L in previous reference literature [8,9,12]. Since common amino acids in nature are generally L configuration, we deduced the amino acid residues in compound **1** were likely to be L. Finally, compound **1** was identified as shown in Figure 1 and named as psammosilenin C.

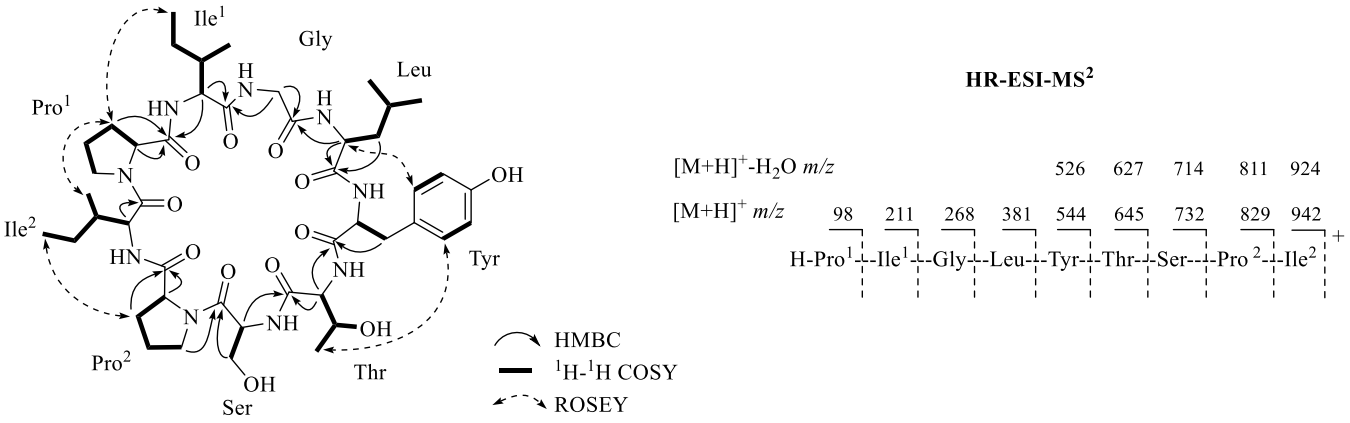

Figure 2. Key 2D NMR correlations and HR-ESI-MS^2 fragmentation of compound **1**

The residual compounds were confirmed as 1-O-(*β*-D-glucopyranosyl)-(2*S*,3*R*,4*E*,8*E*)-2-[(2*R*)-2-hydroxypentadecanoylamino]-4,8-octadecadiene-1,3-diol (**2**) [14], dihydroferulic acid (**3**) [20] and vanillylacetone (**4**) [20] based on NMR spectral data compared with that of the literatures. The NO inhibitory activity of the ethanol extracts and all compounds on RAW 264.7 macrophages in the stimulation of LPS were assessed. The extracts showed inhibitory effects with the IC_{50} value of 75.97 μg/mL. Compounds **1**, **2** and **4** moderately inhibited NO release by LPS-activated macrophages in a dose-dependent manner, with the IC_{50} value of 106.4, 68.1 and 127.4 μM, respectively. Dihydroferulic acid (**3**) has no inhibitory activity. L-NMMA as positive control had an IC_{50} value of 12.0 μM.

Acknowledgments

This work was partially supported by the National Natural Science Fund of China (No. 81960783), Major Yunnan Science and Technology Project (No.202102AA100018), and the Yunnan Fundamental Research Projects (No. 202101AT070111).

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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