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A NEW CYCLIC PEPTIDE FROM BULBS OF *Fritillaria hupehensis*

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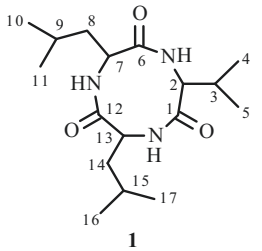
*A new cyclic peptide, cyclo(Leu-Val-Leu) (**1**), along with four known compounds (**2–5**), was isolated from the bulbs of* Fritillaria hupehensis *Hsiao and K.C. Hsia. The structure of compound **1** was elucidated by spectroscopic methods including 1D NMR and 2D NMR analyses as well as HR-ESI-MS techniques.*

Keywords: *Fritillaria hupehensis*, nonbasic constituents, cyclic peptide.

Fritillaria hupehensis Hsiao & K. C. Hsia is a liliaceous plant growing in the southwest district of Hubei Province, China. Its bulbs have been recorded in Pharmacopoeia of the People′s Republic of China as a principal Chinese traditional medicine named "Hubeibeimu." C-nor-D-homo steroidal alkaloids have been isolated from the bulbs of this plant, together with a number of the nonbasic constituents, such as fritillebin C and fritillebin D [1]. In our continuing studies on the nonbasic constituents, a new cyclic peptide named cyclo(Leu-Val-Leu) (**1**), together with four known compounds (**2–5**), was isolated from this plant. In this article, we describe the isolation and structural elucidation of compound **1**. The structures of compounds **2–5** were respectively confirmed as 13β-hydroxy-7-oxoabiet-8(14)-en-19,6β-olide, thymidine, adenosine, and uridine.



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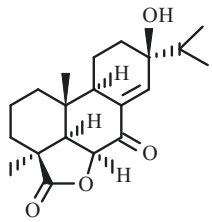

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Compound **1** was obtained as a white amorphous powder. Its molecular formula was determined as $C_{17}H_{31}N_3O_3$ according to the HR-ESI-MS data (*m*/*z* 326.4290 $[M + H]^+$, calcd 326.4294), requiring four degrees of unsaturation. The IR spectrum of **1** showed intense absorption bands for amide N–H at 3300 cm^{-1} and amide C=O at 1650 cm^{-1}. Analysis of the ^1H and ^{13}C NMR data (Table 1) revealed the presence of three carbonyl signals (δ_C 168.9, 168.8 and 167.5), three normal α-amino acid methine carbon resonances (δ_C 31.3, 23.6 and 23.5), and three amide protons (δ_H 8.11, 8.09, and 8.01), indicating its peptide nature [2–5].

^1H–^1H COSY experiment (Fig. 1) constructed the three amino acid residues as a valine and two leucines, which were in correspondence with that of amino acid analysis after complete acid hydrolysis (6N HCl, 110°C, 24 h). These residues accounted for three of the four degrees of unsaturation, indicating that compound **1** is a cyclopeptide. Since compound **1** gave a negative response, but a positive response after its acid hydrolysis, in the ninhydrin test, it must be a cyclopeptide [6].

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TABLE 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) Data of Compound **1** (DMSO-d_6, δ, ppm, J/Hz)

C atom	δ_H	δ_C	C atom	δ_H	δ_C	C atom	δ_H	δ_C
Val			Leu1			Leu2		
1		167.4 (s)	6		168.8 (s)	12		168.9 (s)
2	3.64 (1H, m)	59.2 (d)	7	3.75 (1H, m)	52.3 (d)	13	3.75 (1H, m)	52.4 (d)
3	2.18 (1H, m)	31.3 (d)	8	1.55 (2H, m)	41.3 (t)	14	1.55 (2H, m)	41.7 (t)
4	0.84 (3H, d, J = 6.7)	16.8 (q)	9	1.81 (1H, m)	23.6 (d)	15	1.81 (1H, m)	23.5 (d)
5	0.94 (3H, d, J = 6.7)	18.3 (q)	10	0.85 (3H, d, J = 3.9)	22.9 (q)	16	0.86 (3H, d, J = 3.9)	22.8 (q)
N–H	8.01 (1H, br.s)		11	0.86 (3H, d, J = 3.9)	21.9 (q)	17	0.87 (3H, d, J = 3.9)	22.1 (q)
			N–H	8.09 (1H, br.s)		N–H	8.11 (1H, br.s)	

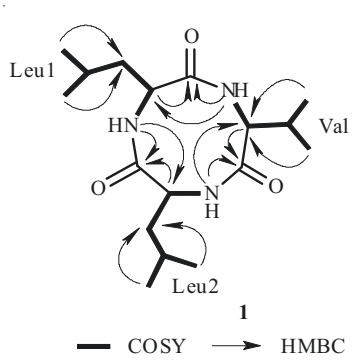

Fig. 1. Key ^{1}H–^{1}H COSY and HMBC correlations of compound **1**.

Evidence of the sequence of these amino acid residues was provided by the ^{1}H–^{1}H COSY and HMBC experiment. HMBC correlations were observed between Val-NH (δ_H 8.01)/Leu1-CO (δ_C 168.8), Leu1-NH (δ_H 8.09)/Leu2-CO (δ_C 168.9), and Leu2-NH (δ_H 8.11)/Val-CO (δ_C 167.4), indicating the peptide fragment Leu2-Val-Leu1.The absolute stereochemistry of the amino acid residues in **1** was confirmed by the advanced Marfey′s method [7]. Compound **1** was subjected to complete hydrolysis with 6 N HCl at 110°C for 24 h in a sealed tube. Each amino acid from the hydrolysate was purified through silica gel TLC and then analyzed by comparing the R_f-values with standard amino acids on chiral-TLC [8]. Consequently, all amino acids were established to be of the L-configuration. Therefore, the structure of compound **1** was assigned as cyclo(Leu-Val-Leu).

EXPERIMENTAL

General Procedures. NMR spectra were recorded on a Bruker AV-500 spectrometer with TMS as internal standard. HR-ESI-MS spectra were measured with an Agilent 1100 LC/MSD TOF mass spectrometer.

Plant Material. The bulbs of *F. hupehensis* Hsiao & K. C. Hsia were obtained from Hubei Institute of Chinese Materia Medica and identified by Peng De Tai, Li Chuan Institute of Chinese Materia Medica, China. A voucher speciman (No. 09-15-2010) was deposited at the Faculty of Pharmaceutical Sciences, Tongji Medical College of Huazhong University of Science and Technology, Wuhan, China.

Extraction and Isolation. The powdered crude bulbs (7.3 kg) of *F. hupehensis* were extracted with MeOH under reflux for three times. After removal of the solvent at reduced pressure, the MeOH extract (560 g) was suspended in water and then partitioned with EtOAc and *n*-BuOH successively. The EtOAc extract (80 g) was subjected to silica gel column chromatography (petroleum ether–EtOAc, 100:0–50:50) to obtain five fractions 1–5. Fraction 2 (8.5 g) was then chromatographed on a silica gel column (petroleum ether–Me_2CO, 20:1–1:1) to afford four fractions (fractions 2.1–2.4). Compound **2** (13.4 mg) was obtained from fraction 2.2 by recrystallization. The *n*-butanol extract (155 g) was chromatographed on a silica gel column ($CHCl_3$–MeOH, 100:0–0:100) to give nine fractions 1–9. The combined fractions eluted with $CHCl_3$–MeOH (fraction 7, 12.5 g) were separated on a silica gel H column ($CHCl_3$–MeOH, 50:1–1:2) to afford seven fractions 1′–7′. Subfraction 3′ (0.15 g) was purified repeatedly by semipreparative HPLC (20% MeOH–H_2O) to give compounds **3** (15 mg), **4** (12 mg), and **5** (10 mg). Subfraction 5′ (0.1 g) was further purified by Sephadex LH-20 column ($CHCl_3$–MeOH, 1:1) to yield compound **1** (3 mg).

Compounds **2–5** were obtained from this plant for the first time. The structures of compounds were respectively determined as 13β-hydroxy-7-oxoabiet-8(14)-en-19,6β-olide (**2**) [9], thymidine (**3**) [10], adenosine (**4**) [11], and uridine (**5**) [12] by comparison with the literature values.

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