Fitoterapia 98 (2014) 53–58

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

New amino butenolides from the bulbs of *Fritillaria unibracteata*

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ARTICLE INFO

Article history:
Received 14 May 2014
Accepted in revised form 8 July 2014
Available online 23 July 2014

Keywords:
Fritillaria unibracteata
Liliaceae
Amino butenolides
Amide alkaloids

ABSTRACT

Five new amino γ-butenolides, fritenolide A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**), along with four known compounds, were isolated from the bulbs of *Fritillaria unibracteata*. Their structures were determined by spectroscopic analysis, including 1D NMR, 2D NMR, HRESIMS, HRESIMS/MS, IR, and CD techniques. All isolates were evaluated for the protective activity on injured hepatocytes and cytotoxic activity on human cancer cells *in vitro*. The unusual amino butenolides were isolated from the Liliaceae family for the first time.

1. Introduction

The genus *Fritillaria* (Liliaceae) is approximately composed of 130 species in the whole world, and mainly distributed in temperate regions of the Northern Hemisphere [1]. The bulbs of several *Fritillaria* plants have been used as a traditional Chinese medicine to relieve coughing for nearly 2000 years in China [2]. Previous phytochemical and pharmacological studies on the genus have led to the isolation of a lot of bioactive secondary metabolites, such as alkaloids, steroids, saponins, and terpenoids [3–7]. In order to search for more active ingredients, we carried out an investigation on the ethanolic extract of the bulbs of *Fritillaria unibracteata* Hsiao et K. C. Hsia, which is one of the sources for "Chuanbeimu" in Pharmacopoeia Commission of the People's Republic of China [2]. This paper describes the isolation, structure elucidation, and bioassays of the isolates. The new compounds **1**–**5** (Fig. 1) are α,β-unsaturated γ-butenolides with an amide unit. It is of interest to note that the unusual amide butenolides have been only reported from marine organisms and fungus before [8–11].

2. Experimental

2.1. General

NMR spectra were recorded on a Bruker-AVIIIHD-600 spectrometer. HRESIMS and HRESIMS/MS were measured with a Waters Synapt G2 Q-TOF HDMS spectrometer. IR spectra were recorded on a Nicolet 5700 FT-IR microscope instrument. Optical rotations were measured with a Perkin-Elmer 341 plus. CD spectra were recorded on a JASCO J-815 CD spectrometer. Column chromatography was performed with silica gel (200–300 mesh, Yantai Institute of Chemical Technology, Yantai, China) and Sephadex LH-20 (Amersham Pharmacia Biotech AB, Uppsala, Sweden). Preparative TLC (0.4–0.5 mm) was conducted with glass plates precoated silica gel GF_{254} (Yantai). HPLC separation was performed on an instrument

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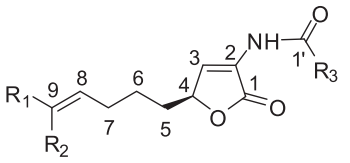
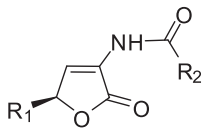
http://dx.doi.org/10.1016/j.fitote.2014.07.009
0367-326X/



1 $R_1 = (CH_2)_8CH_3$, R_2 = H, $R_3 = (CH_2)_{12}CH_3$
2 R_1 = H, $R_2 = (CH_2)_8CH_3$, $R_3 = (CH_2)_{12}CH_3$
3 $R_1 = (CH_2)_8CH_3$, R_2 = H, $R_3 = (CH_2)_{10}CH_3$



4 $R_1 = (CH_2)_{13}CH_3$, $R_2 = (CH_2)_{12}CH_3$
5 $R_1 = (CH_2)_{13}CH_3$, $R_2 = (CH_2)_{10}CH_3$

Fig. 1. Chemical structures of compounds **1**–**5**.

consisting of a Cometro 6000LDS pump and a Cometro 6000PVW UV/VIS detector with an Ultimate (250 × 10 mm) preparative column packed with C_{18} (5 μm).

2.2. Plant material

The bulbs of *F. unibracteata* were purchased from Sichuan Neautus Traditional Chinese Medicine Inc., and were originally collected from Sichuan province, China. Plant identity was verified by Prof. Min Li (Chengdu University of Traditional Chinese Medicine, Chengdu, China). A voucher specimen (FU20111202) was deposited at the School of Pharmacy, Chengdu University of Traditional Chinese Medicine.

2.3. Extraction and isolation

The air-dried bulbs of *F. unibracteata* (5 kg) were extracted three times with 95% EtOH (3 × 30 L, total amount 90 L) for 2 h under reflux. The EtOH extract was concentrated in vacuo to yield a semi-solid (275 g), which was suspended in H_2O (2 L) and then partitioned sequentially with chloroform (6 × 1.5 L) and *n*-BuOH (6 × 1.5 L). The chloroform extract (28 g) was chromatographed on silica gel with petroleum ether–ethyl acetate (100:1, 50:1, 20:1, 10:1, 5:1, 2:1, 1:2, v/v) to afford 27 fractions (Fr.1–Fr.27) based on their TLC patterns. Fr.15 (2.1 g) was applied to Sephadex LH-20 using petroleum ether–$CHCl_3$–MeOH (5:5:1, v/v) as the mobile phase to yield five subfractions (Fr.15-1–Fr.15-5). Eluting with a gradient of petroleum ether–acetone (100:1–4:1, v/v), Fr.15-1 (0.5 g) was separated by column chromatography over alkaline silica gel, to give six subfractions (Fr.15-1a–Fr.15-1f). Fr.15-1a (68 mg) was further purified by preparative TLC (petroleum ether–acetone, 4:1) followed by reversed-phase semi-preparative HPLC (98% MeOH in H_2O) to yield **1** (6.8 mg), **2** (4.6 mg), **3** (2.1 mg), **4** (2.4 mg), and **5** (1.7 mg).

Fritenolide A (**1**): white amorphous powder; $[\alpha]_D^{20}$ −7.3 (*c* 0.05, MeOH); CD (MeOH) 246.5 ($\Delta\varepsilon$ + 1.12) nm; IR ν_{max} = 3330, 2954, 2921, 2851, 1744, 1694, 1654, 1548, 1468, 1347, 1072, 965, 786, 721 cm^{-1}; ^1H NMR and ^{13}C NMR data see Tables 1 and 2; HRESIMS *m/z*: 526.4232 $[M + Na]^+$ (calcd for $C_{32}H_{57}NO_3Na$, 526.4236); HRESIMS/MS *m/z*: 482.4340 $[M - CO_2 + Na]^+$ (calcd for $C_{31}H_{57}NONa$, 482.4338), 316.2253 $[M - C_{14}H_{26}O + Na]^+$ (calcd for $C_{18}H_{31}NO_2Na$, 316.2252), and 272.2354 $[M - CO_2 - C_{14}H_{26}O + Na]^+$ (calcd for $C_{17}H_{31}NNa$, 272.2354).

Fritenolide B (**2**): white amorphous powder; $[\alpha]_D^{20}$ −7.7 (*c* 0.04, MeOH); CD (MeOH) 243.5 ($\Delta\varepsilon$ + 1.11) nm; IR ν_{max} = 3326, 2955, 2921, 2852, 1743, 1692, 1652, 1546, 1465, 1354, 1071, 877, 787, 719 cm^{-1}; ^1H NMR and ^{13}C NMR data see Tables 1 and 2; HRESIMS *m/z*: 526.4242 $[M + Na]^+$ (calcd for $C_{32}H_{57}NO_3Na$, 526.4236); HRESIMS/MS *m/z*: 482.4339 $[M - CO_2 + Na]^+$ (calcd for $C_{31}H_{57}NONa$, 482.4338), 316.2254 $[M - C_{14}H_{26}O + Na]^+$ (calcd for $C_{18}H_{31}NO_2Na$, 316.2252), and 272.2355 $[M - CO_2 - C_{14}H_{26}O + Na]^+$ (calcd for $C_{17}H_{31}NNa$, 272.2354).

Fritenolide C (**3**): white amorphous powder; $[\alpha]_D^{20}$ −6.9 (*c* 0.04, MeOH); CD (MeOH) 244 ($\Delta\varepsilon$ + 0.94) nm; IR ν_{max} = 3330, 2955, 2923, 2853, 1743, 1693, 1654, 1548, 1454, 1053, 965, 798 cm^{-1}; ^1H NMR and ^{13}C NMR data see Tables 1 and 2; HRESIMS *m/z*:498.3926 $[M + Na]^+$ (calcd for $C_{30}H_{53}NO_3Na$, 498.3923). HRESIMS/MS *m/z*: 454.4022 $[M - CO_2 + Na]^+$ (calcd for $C_{29}H_{53}NONa$, 454.4025), 316.2249 $[M - C_{12}H_{22}O + Na]^+$ (calcd for $C_{18}H_{31}NO_2Na$, 316.2252), and 272.2352 $[M - CO_2 - C_{12}H_{22}O + Na]^+$ (calcd for $C_{17}H_{31}NNa$, 272.2354).

Fritenolide D (**4**): white amorphous powder; $[\alpha]_D^{20}$ −6.4 (*c* 0.05, MeOH); CD (MeOH) 244 ($\Delta\varepsilon$ + 0.99) nm; IR ν_{max} =

Table 1
^{13}C NMR data (150 MHz) for compounds **1**–**5** in $CDCl_3$[a] (δ in ppm).

no.	1	2	3	4	5
1	170.0	169.9	170.0	170.0	170.0
2	125.4	125.4	125.4	125.4	125.4
3	129.5	129.4	129.5	129.6	129.6
4	82.2	82.2	82.2	82.3	82.3
5	33.4	33.5	33.4	34.0	34.0
6	24.9	25.1	24.9	25.1	25.1
7	32.3	26.9	32.3		
8	129.0	128.5	129.0		
9	131.8	131.2	131.8	29.3–29.8	29.3–29.9
10	32.7	27.4	32.7		
11–15	29.3–29.8[b]	29.3–29.8	29.3–29.8		
16	32.1	32.1	32.1	32.1	32.1
17	22.8	22.8	22.8	22.8	22.9
18	14.3	14.3	14.3	14.3	14.3
1′	172.1	172.1	172.1	172.1	172.1
2′	37.0	37.0	37.0	37.0	37.0
3′	25.3	25.3	25.3	25.3	25.4
4′–9′			29.3–29.8		29.3–29.9
10′	29.3–29.8	29.3–29.8	32.1	29.3–29.8	32.1
11′			22.8		22.9
12′	32.1	32.1	14.3	32.1	14.3
13′	22.8	22.8		22.8	
14′	14.3	14.3		14.3	

[a] The assignments were based on ^1H–^1H COSY, HSQC, and HMBC experiments.
[b] The ^{13}C NMR signals for methylenes in the aliphatic chains were overlapped between 29.3–29.8 ppm (C-11–C-15 and C-4′–C-11′ in **1** and **2**, C-11–C-15 and C-4′–C-9′ in **3**, C-7–C-15 and C-4′–C-11′ in **4**, C-7–C-15 and C-4′–C-9′ in **5**).