Fitoterapia 109 (2016) 174–178

Contents lists available at ScienceDirect

Fitoterapia

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

Volvalerine A, an unprecedented *N*-containing sesquiterpenoid dimer derivative from *Valeriana officinalis* var. *latifolia*

Peng-Cheng Wang [a], Xin-Hui Ran [a], Huai-Rong Luo [a], Qing-Yun Ma [b], Jun Zhou [a], Jiang-Miao Hu [a,*], You-Xing Zhao [b,*]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People's Republic of China
[b] Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agriculture Sciences, Haikou 571101, People's Republic of China

ARTICLE INFO

Article history:
Received 11 December 2015
Received in revised form 3 January 2016
Accepted 8 January 2016
Available online 11 January 2016

Keywords:
Valeriana officinalis var. *latifolia*
Valerianacae
Sesquiterpenoid
Bisesquiterpenoid

ABSTRACT

Volvalerine A (**1**), a novel *N*-containing bisesquiterpenoid derivative with a dihydroisoxazole ring, and its possible biosynthetic precursor, 1-hydroxy-1,11,11-trimethyldecahydrocyclopropane azulene-10-one (**2**), were isolated from the roots of *Valeriana officinalis* var. *latifolia*. Their structures and relative configurations were identified using spectroscopic data and X-ray crystallography. A plausible biosynthetic pathway for **1** is also presented.

1. Introduction

Disesquiterpenoids (bisesquiterpenoids, sesquiterpenoid dimers) are a fascinating class of natural secondary metabolites that contain 30 carbons, and they are biosynthetically produced from two different or identical sesquiterpenoids [1]. Disesquiterpenoids have attracted much attention from chemists and biologists because of their unique structural features and diverse bioactivities, including anti-HIV [2], cytotoxic [3], and tyrosinase inhibitory activities [4].

The genus *Valeriana* (Valerianaceae) contained more than 250 species of plants worldwide, especially in Europe, North America, and Asia [5]. *Valeriana officinalis*, commonly known as valerian, is the most often used in United States and Europe. It was used as a mild sedative to aid with sleep since ancient Greek and Roman times [6]. Currently, valerian root is a popular alternative to medicinal remedies for insomnia because it is considered safe and gentle. The ethanol extract of valerian roots has been reported to exhibit antidepressant and anti-anxiety effects in both animal models and clinical trials [7,8]. Although valerian has been widely used for a long time and it is still recorded in both United States Pharmacopeia and European Pharmacopeia, the precise active constituents of valerian are still in controversy [6]. Sesquiterpenoids and iridoids are the two major types of compounds in this genus [9–14], and they have been reported to be responsible for the sedative, anxiolytic, and antidepressant activities of valerian in previous studies [6,7,15–17]. Valerenic acid, one of the main valerane-type sesquiterpenoids, is the official standard for the quality control of valerian products in the United States Pharmacopeia [18].

In our continued efforts to chemically investigate the genus *Valeriana*, particularly to discover the active components related to its central nervous system (CNS) effects, a series of sesquiterpenoids and iridoids were isolated [19–23]. In particular, a new type of sesquiterpenoid dimer was obtained from *V. officinalis* var. *latifolia* [19]. Further studies on the roots of this *Valeriana* species led to the isolation of a novel *N*-containing sesquiterpenoid dimer derivative, volvalerine A (**1**), as well as its possible biosynthetic precursor, 1-hydroxy-1,11,11-trimethyldecahydrocyclopropane azulene-10-one (**2**) [24]. Herein, we report the isolation and structural elucidation of compounds **1** and **2**. Compound **1** has a novel *N*-containing bisesquiterpenoid skeleton, and it consists of two norsesquiterpenoid moieties. These two parts are connected via a dihydroisoxazole ring. In addition, the structure of compound **2** was revised on the basis of comprehensive 2D NMR analysis.

2. Experimental

2.1. General experimental procedures

Melting points were obtained using a micromelting point apparatus (model X-4; Shanghai Automation Instrumentation Co., Ltd., Shanghai, China) and were uncorrected. Optical rotations were recorded on a polarimeter (SEPA-300; Horiba Ltd., Kyoto, Japan). UV spectra were collected on a double-beam spectrometer (210A; Shimadzu Co., Kyoto,

* Corresponding authors.
E-mail addresses: hujiangmiao@mail.kib.ac.cn (J.-M. Hu), zhaoyx1011@163.com (Y.-X. Zhao).

http://dx.doi.org/10.1016/j.fitote.2016.01.003
0367-326X/

Japan). IR spectra were recorded on an IR spectrometer (Tensor 27; Bruker, Billerica, MA, USA) using KBr pellets. NMR spectra were measured on a Bruker AV-400 or a DRX-500 spectrometer, with the residual solvent used as the internal standard. ESI-MS and HRESIMS were recorded with a spectrometer (API QSTAR Pulsar I; Applied Biosystems, Foster City, CA, USA). Column chromatography was performed on either silica gel (200–300 mesh; Qindao Marine Chemical Inc., Qingdao, People's Republic of China) or RP-18 gel (LiChroprep, 40–63 μm; Merck, Darmstadt, Germany). The Sephadex LH-20 for chromatography was purchased from Amersham Biosciences (Amersham Biosciences, Inc., Piscataway, NJ, USA). Fractions were monitored by TLC, and spots were visualized by heating silica-gel plates sprayed with 10% H_2SO_4 in EtOH.

2.2. Plant material

The *V. officinalis* var. *latifolia* plants were collected in October 2008 in Badong County, Hubei Province, People's Republic of China. The plant was identified by Professor You-Wei Wang, School of Pharmaceutical Sciences, Wuhan University, People's Republic of China. A voucher specimen (KIB-XC0810) was preserved at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, People's Republic of China.

2.3. Extraction and isolation

The powdered roots of *V. officinalis* var. *latifolia* (14 kg) were extracted using 95% EtOH at room temperature. The solvent was removed by evaporation under vacuum. The residue (3 kg) was suspended in water and partitioned successively with $CHCl_3$ (3 × 4 L) and *n*-BuOH (3 × 4 L). The $CHCl_3$ extract (800 g) was separated using silica gel column chromatography (CC) eluting with petroleum ether–acetone (from 100:1 to 1:1) to yield eight fractions, A–H. Fraction E (15 g) was then repeatedly subjected to CC on silica gel eluting with petroleum ether–acetone (from 10:1 to 1:1) to afford four fractions: Ea–Ed. Fr. Eb (2 g) was separated on a silica gel column, eluted with $CHCl_3$–MeOH (from 100:1 to 5:1) to yield six fractions (Eb1–Eb6). Fr. Eb3 was purified by repeated silica gel columns and semipreparative HPLC (RP-18, MeOH–H_2O, 30%–90%) and TLC to afford **1** (15 mg). Subfraction Eb4 (120 mg) was repeatedly subjected to silica gel CC eluted with $CHCl_3$–MeOH (from 100:1 to 1:1) and purified by Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) to yield compound **2** (12 mg).

2.3.1. Volvalerine A (**1**)

Colorless prism (CH_3OH); mp = 219–222 °C, $[\alpha]^{21.7}_D = -4.83$ (c 0.11, CH_3OH); UV (CH_3OH) λ_{max} (log ε): 203 (3.34), 221 (3.38) nm; IR (KBr) ν_{max} 3441, 2974, 2949, 2923, 2865, 1635, 1461, 1378, 1250, 1106, 1079 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; (+)-ESI-MS: *m/z* 486 $[M + H]^+$; HRESI-MS: *m/z* 486.3584 $[M + H]^+$ (calcd for $C_{30}H_{48}NO_4$, 486.3583).

2.3.2. Crystallographic data of volvalerine A (**1**)

$C_{30}H_{47}NO_4$, MW = 485.69; space group: monoclinic, *P*2 (1); a = 8.562 (3) Å, b = 11.858 (4) Å, c = 14.002 (5) Å, α = 90.00, β = 79.049 (5), γ. = 90.00, V = 1395.7 (9) $Å^3$, Z = 2, d = 1.156 g/cm^3, and crystal dimensions 0.24 × 0.15 × 0.12 mm were used for measurement on a SHELXL-97 with a graphite monochromater, Mo Kα radiation. The total number of reflections measured was 9674, of which 5108 were observed, $I > 2\sigma(I)$. Final indices: $R_1 = 0.0738$, w$R_2 = 0.1325$. The crystal structure of compound **1** was solved by direct method SHLXS-97 (Sheldrick, 1990) and expanded using the difference Fourier technique, refined by the program SHLXL-97 (Sheldrick, 1997), and the full-matrix least-square calculations. Crystallographic data for the structure of compound **1** have been deposited with the Cambridge Crystallographic Data Centre (deposition no. CCDC 919911).

2.3.3. 1-Hydroxy-1,11,11-trimethyldecahydrocyclopropaneazulene-10-one (**2**)

Colorless powder, $[\alpha]^{16.8}_D = +15.00$ (*c* 0.08, CH_3OH); UV (CH_3OH) λ_{max} (log ε): 203 (2.93) nm; IR (KBr) ν_{max} 3440, 2984, 2946, 2865, 1737, 1452, 1374, 1161, 1124 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; (+)-ESI-MS: *m/z* 245 $[M + Na]^+$; HRESI-MS: *m/z* 245.1511 [M + Na] + (calcd for $C_{14}H_{22}O_2Na$ 245.1517).

2.4. AChE inhibitory activity

S-Acetylthiocholine iodide, *S*-butyrylthiocholine iodide, 5,5′-dithiobis-(2-nitrobenzoic) acid (DTNB; Ellman's reagent), tacrine, AChE, and butyrylcholinesterase derived from human erythrocytes (Sigma Chemical Company, St Louis, MO, USA) were used. Acetylthiocholine iodide (Sigma Chemical Company) was used as substrate in the assay.

Table 1
^1H and ^{13}C NMR data of **1** and **2** in $CDCl_3$.

1						2		
Position	δ_C[a]	δ_H[b] (*J* in Hz)	Position	δ_C[a]	δ_H[b] (*J* in Hz)	Position	δ_C[a]	δ_H[c] (*J* in Hz)
1	56.2	1.94, m	1′	58.1	1.89, m	1	54.6	2.05, m
2	24.7	1.68, 2H, m	2′	25.0	1.74, m	2	22.1	1.60, m
					1.80, m			2.14, m
3	33.9	1.33, m	3′	36.2	1.81, 2H, m	3	37.3	2.13, m
		1.58, m						2.34, dd (16.5, 8.4)
4	84.2		4′	101.4		4	220.6	
5	47.6	1.47, m	5′	47.3	1.36, m	5	48.0	1.41, t (10.8)
6	28.3	0.36, dd (11.0. 9.5)	6′	27.9	0.89, m	6	28.6	0.48, t (9.5)
7	27.0	0.62, m	7′	27.0	0.62, m	7	26.4	0.66, m
8	20.3	1.80, m	8′	20.1	1.80, m	8	20.2	0.81, m
		0.86, m			0.86, m			1.90, m
9	44.8	1.53, 2H, m	9′	44.6	1.72, 2H, m	9	44.4	1.61, m
								1.76, m
10	75.2		10′	75.4		10	74.6	
11	20.3		11′	20.4		11	19.3	
12	16.2	0.95, s	12′	16.8	1.07, s	12	28.3	1.05, s
13	28.7	0.96, s	13′	29.2	1.01, s	13	16.1	1.00, s
14	20.4	1.13, s	14′	20.8	1.17, s	14	20.4	1.13, s
15	107.9	5.58, d (2.6)	15′	166.1	7.56, d (2.6)			

[a] Recorded at 100 MHz.
[b] Recorded at 500 MHz.
[c] Recorded at 400 MHz.